AQUACULTURE OF MILKFISH (*CHANOS CHANOS*): STATE OF THE ART

Edited by

Cheng-Sheng Lee
Malcolm S. Gordon
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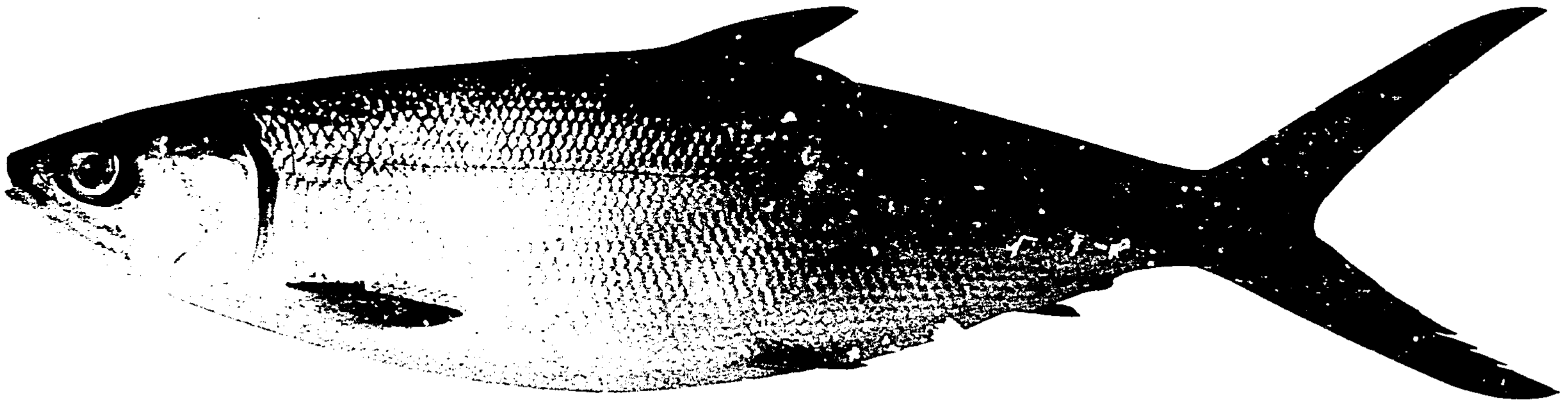

Chanos chanos (Forskål)

(Courtesy I-Chiu Liao, Tungkang Marine Laboratory)

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PREFACE

STATEMENT OF THE PROBLEM

The milkfish is one of the most extensively farmed marine bony fishes on earth. Despite its extensive culture, milkfish remains one of the least well known and understood of the major finfish species.

The prime market size for milkfish throughout most of the Far East is about 300-400 g, meaning fish usually less than one year old. Milkfish do not reach sexual maturity, however, until 5 to 7 years of age. Thus, milkfish farming has been a traditional industry with little emphasis on producing sexually mature, reproductively active fish in captivity.

The traditional milkfish industry depends totally on an annual restocking of farm ponds with fingerlings grown up from wild-caught fry. As a result, the industry suffers from regional, seasonal and annual variations in fry availability. These variations are generally unpredictable, and may be quite large over short periods of time.

A few fish farmers and, more recently, some scientific aquaculturists, have occasionally kept milkfish in captivity until they matured reproductively. Only in the past several years have a few of these efforts resulted in spontaneous spawnings by a few fish. Why these few spawned remains unknown. The culture conditions under which the spawnings occurred did not differ substantially from conditions that did not result in spawning.

Thus, the central problem [illegible] by the international milkfish industry is: What must be done to produce a reliable, adequate, high quality supply of milkfish fry that is not subject to large unpredictable variations in time and space?

WHY THIS BOOK?

This book is meant to provide a complete and up-to-date summary of the most recent work (through December 1985) that goes a substantial distance toward answering this question. As its title indicates, the book is also as complete a statement and summary of current knowledge (also through December 1985) of the milkfish and the milkfish industry as is possible. It is a guide to traditional knowledge, to the results of past technical and scientific research, and to the most recent research.

This book is written for a general audience, but it contains much of the technical information needed by engineers, scientists and fish farmers who wish to make use of the latest information in their own milkfish farming or research projects. This book is probably not the final word on milkfish, but the editors and authors hope it will provide a solid and lasting foundation for future work.

This book is an important component of a major research and development project on milkfish reproduction financed primarily by the U.S. Agency for International Development (AID). This project is being conducted jointly by the Oceanic Institute (OI), Hawaii; the Tungkang Marine Laboratory (TML), Taiwan; and the Aquaculture Department, Southeast Asian Fisheries Development Center (SEAFDEC), Philippines. There is ongoing communication and cooperation between this project and a related program at SEAFDEC that is financed by the International Development Research Centre (IDRC) of the Government of Canada.

STRUCTURE OF THE BOOK

The book is a compilation of chapters written by milkfish experts, most of whom have studied and worked with the species for many years. The authors were chosen by the senior scientific editor, C.S. Lee, in consultation with AID staff and members of the AID project's Technical Advisory

Group (TAG). Each chapter has been refereed by other scientists familiar with the subject matter covered. Revisions were based on these reviewers' comments. Final review and editing of the chapters was done by the scientific editors.

The book opens with a chapter on milkfish biology, summarizing what is known about milkfish in the wild, and discussing various topics not covered elsewhere in the book. Chapters on genetic variation within the species and on reproduction follow. Succeeding chapters describe the traditional industry, from fry capture, distribution and rearing through growth of the fish to market size. Detailed descriptions are given of culture practices in different countries; aspects of pond design and management; nutrition; and common diseases. The final chapter considers the economics of milkfish farming. The book ends with a summary which highlights integral points of the previous chapters and indicates important directions for both near- and long-term future research.

OTHER RECENT PUBLICATIONS ON MILKFISH

Several major publications focusing on milkfish have appeared in recent years. A bibliography of 1,050 papers and reports was recently issued by the Library of the Aquaculture Department, SEAFDEC (SEAFDEC, 1983). Useful reprints of many important original papers are included in the two volumes edited by Benedicto-Dormitorio (1983). Two book-length sets of papers delivered at major symposia devoted to milkfish are: Juario et al. (1984) and Lee and Liao (1985).

As is often the case when the subject of review is a commercially-important organism, many of the literature sources cited in this book are technical reports issued by a variety of research laboratories and/or government agencies. Generally, these have not been published and have rarely been refereed. These papers are usually photocopied or otherwise informally duplicated and are frequently almost impossible to obtain. The Library of the Aquaculture Department, SEAFDEC, is probably the best source for most such documents.

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REFERENCES

Benedicto-Dormitorio, A. (Ed.). 1983. A compilation of SEAFDEC AQD technical papers on milkfish and other finfishes. Information Services Office, Aquaculture Department, SEAFDEC, Iloilo, Philippines. 2 vols.

Juario, J.V., R.P. Ferraris and L.V. Benitez (Eds.). 1984. Advances in Milkfish Biology and Culture. Island Publ. House, Inc., Metro Manila, Philippines.

Lee, C.S. and I.C. Liao (Eds.). 1985. Reproduction and Culture of Milkfish. Oceanic Institute, Hawaii and Tungkang Marine Laboratory, Taiwan.

SEAFDEC. 1983. Milkfish bibliography. Library, Aquaculture Department, SEAFDEC, Iloilo, Philippines.

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1-1. INTRODUCTION

The milkfish (see Frontispiece) is an unusual fish. It is anatomically unusual. It is unusual with respect to its evolutionary position and relationships. It is unusual in both its extremely wide geographic distribution and its ability to tolerate, even to thrive under, very wide ranges of environmental conditions. It is unusual in its life history, which is in large part the basis for its suitability as a farm animal.

This chapter provides an overview of the ways in which the milkfish is unusual, as well as many aspects of its biology that are not. It describes what is known about the biology of milkfish in the wild. We emphasize milkfish as organisms functioning in natural environments. We cite only those published papers and reports we consider most significant for each topic covered. Cross references are provided to other chapters in the book that present important information about milkfish biology. This chapter also includes some miscellaneous information that is not covered elsewhere in the book.

Schuster (1960) provided the first complete overall review and summary of knowledge of the milkfish and is the foundation upon which this chapter has been built.

1-2. MILKFISH AND PEOPLE

When and how humans first encountered milkfish is unknown. The species is widely distributed throughout the tropical Indo-Pacific Oceans, and is often abundant (see Section 1-5). The origins of the traditional milkfish industry are also unknown, but records indicate that pond culture of the species dates back to about 700 years ago in Indonesia (Ronquillo, 1975), to at least 400 years ago in Taiwan and the Philippines, and probably to at least 300 years ago in Hawaii (Section 8-1; Ling, 1977; Johannes, 1981).

As a widely eaten and widely farmed fish, the milkfish is well known to people in many different countries. It is not surprising that the species is known in different regions by a variety of different common names (Table 1).

Scientific knowledge of the milkfish began with its description as a type of mullet by the Danish biologist Petrus Forskal in 1775, based upon preserved specimens

Table 1. Some Vernacular Names for the Milkfish [1]

Country/Region	Name/Phonetic Pronunciation	English Literal Translation or Life History Stage if Known
USA	Milkfish	All stages
Japan	(Sabahii)	All stages
Taiwan-Fukien	(Sahahii)	Lice-eye Fish
Central Taiwan	(Masaba)	Linen Lice-eye Fish
South Taiwan	(Hi Tsu Hii)	Feeding on Sea Grass Fish
China (Mandarin)	(Su Mu Yii)	What You Call It Fish
Philippines		
(Tagalog)	Sabalo	Mother Fish (Large Wild Fish)
(Tagalog)	Bangus	(Small Fish)
(Mindoro)	Banglis	(all stages)
(Visayas)	Bangrus	(Small Fish)
Other Areas	Awa, Banglot, Betel, Bangos, Bangilis, Laon, Lumulukso	(all stages)
North Borneo	Belanak Sembawa	
Indonesia	Bandeng, Banding, Bolu, Baulu	
Malaysia	Bandang, Jangas	
Viet Nam	(Ca Mang)	
Thailand	(Pla Nuan Chan, Nuan Chan Tale, Chalin, Tu Nam Cut, Dok Mai)	
India	(Tullu Caudal, Palai Meen, Pala Kendai, Palmeen, Tullu-Kendai, Paala Bonta, Tullu, Poomeen, Hoomeenu)	
Ceylon	Hyder's Fish (Vaikka, Pal Meen)	
Saudi Arabia	(Anged, Anget)	
Australia	Salmon Herring, White Mullet	
Palau	Mesekelat, Chaol	
Fiji	Yawa, Awa, Luya	
Society Islands	Ava, Awa	
Hawaii	Pua Awa	(Juveniles)
	Awa-'Ava	(Medium Size)
	Awa	(Commercial Size)
	Awa-Kalamoho	(Large Size)
Mexico	Sabalo, Sabalote	

[1] This list is clearly incomplete. The names given are from many sources, about half from Schuster (1960). Most names given refer to juveniles and subadults; other growth stages probably have other names. The names are also not necessarily always exclusively referable to Chanos, as fish categories recognized by vernacular names do not always coincide with scientific, systematic categories.

(dried skins) from the Red Sea. Subsequently a number of other ichthyologists also described the species and gave it various different names. Herre (1936) provides this synonymy:

Mugil chanos Forskal 1775
Mugil chani Bonnaterre 1788
Mugil salmoneus Bloch and Schneider 1801
Chanos arabicus Lacepede 1803
Palah bontah Russell 1803
Lutodeira indica van Hasselt 1823
Lutodeira chanos Ruppell 1828
Cyprinus (Leuciscus) palah Cuvier 1829
Leuciscus ceylonicus Bennet 1832
Leuciscus (Ptycholepis) salmoneus Richardson 1843
Chanos mento Cuvier and Valenciennes (C & V) 1846
Chanos lubina C & V 1846
Chanos chloropterus C & V 1846
Chanos nuchalis C & V 1846
Chanos orientalis C & V 1846
Chanos cyprinella C & V 1846
Chanos salmoneus C & V 1846
Lutodeira salmonea Richardson 1848
Butirinus argenteus Jerdon 1849
Butirinus maderaspatensis Jerdon 1849
Chanos pala Cantor 1849
Chanos tolo Cantor 1849
Chanos indicus Bleeker 1859
Chanos (Lutodeira) chanos Gunther 1866
Lutodeira chloropterus Playfair 1867
Chanos chanos Klunzinger 1871

The presently accepted scientific name for the species is *Chanos chanos* (Forskal). We adopt the spelling of *Forskal* for the original author on the basis of linguistic advice from native speakers of Swedish.

1-3. EXTERNAL APPEARANCE AND ANATOMY

1-3.1. EXTERNAL APPEARANCE

The overall appearance of the adult milkfish is shown in the Frontispiece. A biologically minor point that has major significance for both the traditional industry and for research efforts directed toward breeding milkfish in captivity (see Chapter 4) is that the sexes are externally indistinguishable in immature fish. There are no readily visible, reliable external features permitting identification of the sexes until the fish reach quite large sizes, become sexually mature, and enter breeding condition (Chaudhuri et al., 1976; but see Section 4-2.1).

Herre and Mendoza (1929) give the following detailed description of the genus Chanos:

> "Body elongate, moderately compressed and pointed at both ends, with a broad rounded abdomen; head naked, with a large transparent imperforate adipose eyelid covering eye and side of head; trunk covered with firm, closely adherent, relatively small or medium-sized silvery scales; dorsal and anal each with broad basal sheath; pectoral with a pointed axillary scale above; small scales extend upon the caudal; snout depressed; mouth small, terminal, transverse and toothless; lower jaw slightly occluded, with a symphysial tubercle, which fits into a notch in the upper jaw between the premaxillaries; the maxillaries short and broad with no supplemental bone; premaxillary joined at the upper anterior edge of maxillary; ventrals well developed, opposite the anterior half of the dorsal; the anal very much smaller than dorsal and placed very far back; caudal large, deeply forked; lateral line straight, each scale with a single tube; gill membranes entirely united below and free from the isthmus; four long branchiostegals; pseudobranchiae well developed;

gillrakers exceedingly fine, numerous and close set in two diverging rows; an accessory branchial organ in a cavity behind the gill cavity proper; air bladder divided by a constriction into an anterior and posterior portion; mucus membrane of the oesophagus raised into a spiral fold; pyloric appendages numerous, intestine very long and with many convolutions, the peritoneum black."

Herre (1936) gives this technical description of juveniles and adults of the species Chanos chanos:

"Dorsal II, 12 to 14; anal II, 8 or 9; pectoral I, 15 or 16; ventral I, 10 or 11; there are 75 to 85 scales in the lateral line to the caudal base, plus 5 to 11 more on the latter; there are 12 or 13 scales above and 10 or 11 below the lateral line; vertebrae 26 + 18 = 44. The elongate, compressed body becomes thicker with age, the head relatively shorter and broader in large, old specimens; the depth is 3.5 to 3.75 times, the head 3.5 to 3.8 times in the length; the large eye is covered with a thick adipose lid, 2.75 to 3.5 times in the head, usually 3.25 to 3.5; the broad snout is usually three-fourths of the eye; the interorbital is broad, flat, equal to the eye in small or medium-sized specimens; in very large or mature specimens it becomes convex and a fourth or half more than the eye; the origin of the dorsal is nearer to the caudal base than to the tip of the snout; the large, scaly, basal sheath of the dorsal is elongated posteriorly; the anterior dorsal ray is falcate, 1.3 to 1.4 times in the head; the anal is much smaller than the dorsal, its height 3.36 to 5.9 times in the head; the very large, deeply forked caudal is 2.44 to 2.88 times in the length; the pointed pectoral is 1.6 to 1.85 times, the ventral 1.85 to 2.15 times in the head.

"The color in life is brilliantly silver over all, brilliant glossy blue or bluish olive above, the top of the head yellowish olive, the sides whitish, white beneath. There are opalescent and golden glints on the sides of the head; the dorsal is yellowish, the pectoral, anals, and ventrals more or less yellow; the caudal is gray or colorless, with a blackish posterior margin. Sometimes the inside of the pectoral and ventrals is dusky or black. The color in alcohol is bluish above, the sides merging gradually into white beneath, a brilliant silver luster over all; the fins are all whitish, or the dorsal and caudal dusky; the eye is more or less deep reddish yellow.

"Adult Chanos are from about three-fourths of a metre to a metre and a half in length, exclusive the caudal fin. Specimens of this size differ much from the young in the shape of the top of the head and the anterior portion of the body. Sometimes specimens a metre or more in length are bulkier in proportion and at the shoulder girdle may be as large as a man's thigh. The top of the head is flat in the young and becomes convex in the adult."

Fowler (1959) provides some additional data on external anatomy, specifically for milkfish from Fiji:

"Depth 2 1/2 to 4 3/4; head 3 1/4 to 4 1/2, width 1 4/5 to 2 1/8. Snout 3 1/5 to 4 in head; eye 3 1/2 to 7, greater than snout, 1 1/4 to 1 2/5 in interorbital, covered with large adipose lids; maxillary not quite reaching eye, length 4 to 4 1/4 in head; interorbital 2 2/5 to 3 1/8, low or only slightly convex. Gill rakers 147 to 160 + 107 to 165, fine, extremely slender, 2 1/2 to 3 in gill filaments.

"Scales 78 to 80 in lateral line to caudal base and 8 to 11 more on latter; 12 or 13 scales above, 9 to

11 below, 30 to 46 predorsal. Scales with 31 to 51 horizontal parallel striae, ending in slender points; basal notch well developed, with rather coarse circuli 12 to 15 or fine.

"D. IV to VI, 9, I to 12, I, fourth to sixth ray 1 1/4 to 1 2/5 in total head length; A. III, 6, I to 8, I, third simple ray 3 1/8 to 3 3/4; at least depth of caudal peduncle 2 1/2 to 3; pectoral 1 2/5 to 1 2/3; ventral 1 3/4 to 2 1/8; caudal 2 3/4 to 3 1/8 in rest of body.

"Dull olive brown, paler to whitish below. Sides and under surfaces bright silvery white. Dorsal and caudal pale brownish to grayish, also pectoral above, fins otherwise whitish."

Detailed descriptions of the morphology of embryonic, larval and postlarval (fry) stages of development are given in Sections 5-2 and 5-3. Buri et al. (1981) also summarize considerable information on osteology and pigmentation of wild larvae.

1-3.2. MORPHOLOGICAL VARIATION

As the descriptions just given indicate, there is limited meristic and morphological variability within the species. Winans (1985) made a detailed study of geographic variation in the species, studying six meristic and 19 morphometric characteristics. He obtained a substantial series of specimens from 15 locations across the Pacific Ocean, from the Philippines, along the equator to Tahiti, and north to Hawaii. Over this large distance, he found only small,though statistically significant, differences in body shape; meristic characters did not vary. Fish from the Philippines on average had proportionally smaller heads and larger tails than fish from either the equatorial Pacific or Hawaii. Even these small differences, however, may have partly resulted from differences in sizes of specimens from the different regions. Further, the differences were not all consistent between the specific localities within each region.

Winans (1985) concludes that a more extensive study will be necessary to determine whether or not there is indeed significant consistent morphological variation between milkfish populations across the Pacific. If such variation does exist, its manifestations are subtle.

Senta and Kumagai (1977), in a paper not cited by Winans, provide additional support for Winans' conclusion. They ounted vertebral numbers in 2,497 milkfish fry collected from nine localities in India, Thailand, Indonesia, the Philippines, Taiwan and French Polynesia (Rangiroa Atoll). The fry were all large enough (over 11 mm total length) to have ossified their vertebral columns sufficiently to permit reliable counts of numbers of vertebrae. Careful statistical analysis of the results indicated that at least four groups were reliably distinguishable: India, Thailand, Indonesia-Philippines-Taiwan, and Polynesia. The differences between these groups were small (means ranging from 43.08 for the Indian group to 43.82 for the Polynesian group). There was some indication of the possible existence of smaller scale local variations within the Indonesia-Philippines-Taiwan region. The general trend was for vertebral numbers to increase from west to east.

Winans (1980) also carried out an analysis of genetic variability within the same milkfish populations described in his 1985 paper. The results of that work, and other information on the population structure of the species, are summarized in Chapter 2.

On rare occasions, individual milkfish are encountered which are markedly shorter and deeper-bodied than "typical" fish (Schuster, 1960). These specimens are found across the Pacific, both in the wild and in ponds. They have the same numbers of vertebrae as "typical" fish. Such fish are probably the basis for the low values of depth/length ratios cited above for Fijian fish by Fowler (1959). The causes of this phenomenon are unknown. Schuster (1960) also mentions reports of milkfish in Indonesia with greatly elongated dorsal, pectoral and caudal fins.

1-3.3. INTERNAL ANATOMY

With respect to internal anatomy, Rabor (1938) provided a detailed description of the adult skeletal system. Buri and Motoh (1980) greatly expanded upon and refined Rabor's description of the adult skull. Sections 5-2 and 5-3, and the paper by Buri et al. (1981) describe the development of the internal structures of embryos, larvae and postlarvae (fry).

The anatomy of the alimentary tract was described by Bridge and Boulenger (1910; see also Section 7-2). The alimentary tract of the juvenile to adult milkfish possesses at least two structural features that are unusual: 1) the paired pharyngeal organs located at the back of the pharynx, just forward of the start of the esophagus; and 2) the spirally folded walls of the upper esophagus. They may be related to the predominantly algal diet of these stages. Exact functions of these features are, however, uncertain and to some degree controversial.

Kapoor (1954), Chandy (1956), Chandy and George (1960), and Kuwatani and Kafuku (1978) give detailed descriptions of both the anatomy and histology of the pharyngeal organs [similar appearing structures occur in comparable anatomical positions in a variety of other species of fishes as well (Nelson, 1967; Kapoor et al., 1975); other names used by anatomists for these structures include: epibranchial organs, accessory branchial organs, Kiemenschnecke (German: "gill coils"), and crumenal organs (Moyle and Cech, 1982)]. These organs consist of a pair of muscular outpocketings of the posterior roof of the pharynx; each organ is shaped like a curved horn. The series of posterior gill rakers of the lower parts of the fourth gill arches and rakers on the fifth ceratobranchial bones extends well into the tubular openings of the organs. The epithelial linings of the organs have the histological appearance of mucous secreting tissues.

The functions of these organs are unknown. Chandy (1956), Chandy and George (1960), and Kuwatani and Kafuku (1978) observed algal cells and protists in their lumens (see also Section 5-4.5). They speculate that at least part of the planktonic food eaten by the fish is mixed with mucous in or from the organs as it is swallowed. Moyle and Cech (1982), however, noting the muscular walls of the organs, and the numerous gill rakers present in their lower sections, postulate that they act as a kind of pharyngeal mill to help break up swallowed food particles. Neither hypothesis seems to have any experimental basis. Neither hypothesis includes possible biochemical roles for the organs in early stages of digestion. Chiu and Benitez (1981) and Gorriceta (1982) found amylase and lipase activities in these organs (see Section 7-5).

Chandy (1956) also studied the spirally folded walls of the upper esophagus. She points out that this structure appears to be unique among fishes. Spiral folds (sometimes called spiral valves) are fairly widely found in the posterior sections of the intestines of many fish groups, especially chondrichthyans, dipnoans and various primitive teleosts. They are usually considered to be adaptations for increasing efficiencies of absorption of digested food materials. No other species known has such structures at the anterior end of the gut.

The milkfish esophagus is a narrow tube with fairly thick walls. The mucosa of these walls is raised into a series of 20-22 closely arranged folds in the form of a spiral coil extending the entire length of the esophagus. Each fold is about 1 mm high, with both surfaces raised into obliquely arranged alternate ridges and furrows. The ridges are thickly covered with small papillae.

Histological studies of the folds led Chandy (1956) to conclude that their principal function is the production of mucous. She postulates that the spiral structure facilitates the mixing of planktonic food with this mucous to form a "food cord" that moves rapidly down to the stomach. In the absence of additional information, particularly concerning possible enzymatic activities associated with the mucous, it is not presently possible to say more.

1-4. EVOLUTIONARY POSITION AND RELATIONSHIPS

Systematic biologists classify the milkfish as unique. It is the only species in its genus, and its genus (Chanos) is the only one in its family of true (euteleostean) bony fishes (Chanidae). The Chanidae is one of four families of unusual fishes comprising the order Gonorynchiformes. The members of this order are quite varied in their appearance, but are considered to be evolutionarily closely related to each other, based on a variety of anatomical, especially skeletal, features. Two of the other three families in the order are also monotypic (one species in one genus each). One of these monotypic families is entirely marine, occurring, like the milkfish, in the Indo-Pacific region. The other two families are both found in fresh water in Africa. The milkfish is the only member of the group that is euryhaline, occurring in fresh water, brackish water and marine habitats. Fossil fishes considered to be evolutionarily close to the ancestral forms of the milkfish have been found in freshwater deposits in North America dating from the middle Eocene (about 50 million years ago) (Greenwood et al., 1966; Rosen and Greenwood, 1970; Nelson, 1976).

The most distant reasonably traceable evolutionary ancestors of the milkfish were probably salmon-like (salmoniform) fishes. As time went on, the descendants of these fishes evolved in three major directions. One group evolved to become the present day salmoniform fishes (order Salmoniformes and related orders). A second group evolved to become one of the major groups of primarily fresh water fishes of the world, the carp-like fishes (Order Cypriniformes), including, as a large subgroup, the characins (Suborder Characoidei). The third group, which today shows substantial morphological affinities to both the salmoniformes and the characins, is the Gonorychiformes. The milkfish, therefore, while a representative of a distinctive group of fishes, possesses a variety of structural features that show its relationship to both salmon-like and carp-like fishes. Its general appearance is similar to that of many characins (Greenwood et al., 1966; Rosen and Greenwood, 1970; Nelson, 1976).

We must note that the picture just described is not agreed to in all respects by all students of the relationships of the groups of bony fishes. Fink and Fink (1981) also made a detailed study of the same groups considered by the authors just cited. They used a different method for analyzing their data (cladistic analysis). Among other results they concluded that: 1) <u>Chanos</u> is indeed unique, belonging to its own subgroup of the Gonorynchiformes, deriving from a common ancestor that gave rise to the entire order; 2) the Gonorynchiformes as a whole are a separate group from, but derive from a common ancestor with, all of the orders of bony fishes possessing a particular set of bony elements in their anterior skeletons called the Weberian apparatus; 3) the order of fishes most closely related to the Gonorynchiformes is the Cypriniformes, the carp-like fishes, <u>not</u> the characins.

1-5. DISTRIBUTION AND ECOLOGY

1-5.1. SPATIAL DISTRIBUTION AND ECOLOGY

Milkfish occur throughout virtually the entire tropical Indo-Pacific Ocean. They are known from the Red Sea and the coast of East Africa to the west coast of Central America (as eastern and western limits), to southern Australia, New Zealand, and Norfolk Island (as southern limits), and southern Japan, Hawaii, and Mexico (as northern limits). They are particularly abundant in southeast Asian and western Pacific waters (Fowler, 1959; Schuster, 1960; Rabanal and Ronquillo, 1975).

They occur in a variety of habitats, including coastal waters, estuaries, mangrove flats, brackish lagoons, tidal flats, rivers and streams. Schools of adult milkfish regularly spend some time each year in littoral waters. In Madagascar, specimens ranging from 17-88 cm in length and 0.6-5.6 kg in body weight have been found in large rivers near the coast. Others were observed 150 km off shore (Therezien, 1976). Milkfish have also been found in small streams and along the coast in southern Japan (Senta and Hirai, 1980).

We do not know where in the sea the vast majority of the wild subadult and adult populations of milkfish spend most of their time. Adult fishes are only rarely and inadvertently captured by most open sea fisheries. Substantial numbers of adult fishes occur in fresh waters in lakes of various sizes in the Philippines, Indonesia, Papua-New Guinea, etc. (Rabanal and Ronquillo, 1975).

Schuster (1960) and Buri et al. (1981) provide detailed discussions of what is known about the nature and locations of milkfish spawning areas and the different geographic distributions of the major growth stages of the fish (eggs, larvae, postlarvae (fry), juveniles, subadults and adults). Note, however, that virtually all of the work done on these subjects was done in the western Pacific only. Sections 3-5 and 8-2 summarize specific aspects of these data. Sections 5-2 and 5-3 present detailed descriptions of embryonic and larval development. Selected points relevant in the present context are:

1) Spawning usually occurs at moderate distances off shore, in clear waters from 10-40 m deep, often over sandy or coral bottoms. Where depths increase gradually with distance from shore, spawning is likely to occur further out. The few known direct observations of spawning aggregations (Johannes, 1981) indicate that spawning often occurs near the sea surface.

2) Eggs initially float, but soon start slowly sinking. Hatching occurs after about 24 hours, apparently at depths down to several meters. Yolk-sac larvae are also slightly negatively buoyant, but their distribution in the water column is unknown (see Section 5-3 for a description of larval behavior in the laboratory). The yolk-sac stage lasts about three days, at the end of which the fish are 6-8 mm total length (TL). Both eggs and yolk-sac larvae seem to be almost completely planktonic, thus are distributed primarily passively by water movements.

3) Exogenously feeding postlarvae (henceforth "fry") less than 10 mm TL are almost unknown from nature. Fry from 6-10 mm TL in the laboratory become progressively more active swimmers and feed vigorously (Section 5-3). It is this size range which makes the first major migration, from the offshore spawning grounds to the littoral aggregation areas that are the sources of fry for the traditional industry. A variety of lines of evidence cited by Buri et al. (1981) make it probable that this migration is primarily an active, oriented set of movements.

4) Fry 10-16 mm TL are 2-3 weeks old. They appear suddenly and in large numbers in clear shallow water close

inshore, usually over sandy bottoms. They avoid turbid, muddy waters, muddy or rocky bottoms, and are not found in their usual areas on stormy days or days directly following storms. In their favored areas, they are often present in huge numbers. Fry in this size range form the basis for the traditional industry, and are actively collected wherever milkfish farming is practiced. They are rapid swimmers with considerable endurance.

5) The transition from fry to juvenile stages takes place at 4-5 weeks of age, after the fry have left the fry aggregation areas. Where wild fry go and what they do during this interval are again unknown. Postmetamorphotic juveniles are next seen in significant numbers in coastal estuaries, lagoons and swamps. Fully metamorphosed fish can be as small as 14 mm TL. Along with the morphological metamorphosis, several other metamorphoses also occur, most notably in nutrition and behavior. Fry are primarily nonschooling, selective planktivores while juveniles are primarily benthic algal filter feeders that travel in schools. Juveniles are highly euryhaline, and enter fresh water streams and lakes.

6) At highly variable ages and equally variable sizes, juveniles make another transition, to subadults. Most subadult fish leave the inshore, protected environments they have occupied and return to the ocean. Some remain in fresh waters, even though not land locked. Very little is known of their distribution in the sea, though they are often caught over reef flats and often enter estuaries on daily feeding migrations. Some have been caught as far as 20 km at sea. Subadults are strong, active, rapid swimmers, clearly capable of traveling long distances.

7) Aquarium- or pond-reared milkfish become sexually mature after 5-6 years (see Chapters 3 and 4). Adult wild milkfish are distributed and occur as was described at the beginning of this section. The largest wild milkfish known

was 1.7 m TL. The oldest milkfish known in captivity was 12 years of age. Age structures of wild milkfish populations are unknown.

Chapter 9 summarizes what is known about the parasites and diseases of milkfish. The ecological importance of predation, parasites and diseases to wild milkfish remains to be determined.

1-5.2. TEMPORAL DISTRIBUTION

Published information on seasonal occurrences of all life history stages of the milkfish appears restricted to either the low latitude tropics or the subtropical northern hemisphere. We assume that seasonal variations in the limited regions of the subtropical southern hemisphere where the species occurs (e.g., southern Australia, southern Madagascar) are appropriately adjusted to the southern hemisphere seasons.

In tropical areas, milkfish seem to be present year round, though significant variations in abundance may occur regionally and not be known. In the subtropics, milkfish are primarily summer visitors (Rabanal and Ronquillo, 1975); winter and early spring records from Japan appear to have been mostly of fish trapped in ponds of various kinds (Senta and Hirai, 1980).

The timing of milkfish spawning in different regions is highly variable (see Sections 3-5.2 and 6-10; Johannes, 1981; Kumagai, 1984). Based primarily on inferences made from both the occurrence of fry in aggregation areas exploited by the traditional industry and laboratory studies of embryonic and larval development, spawnings probably occur most often around new and full moon phases, most often at night (probably after midnight), and, in most regions, in one or two seasonal peaks (April - July, September - November). Figure 1 illustrates the range of seasonal variation. Note that spawning seems to occur in Tahiti all year.

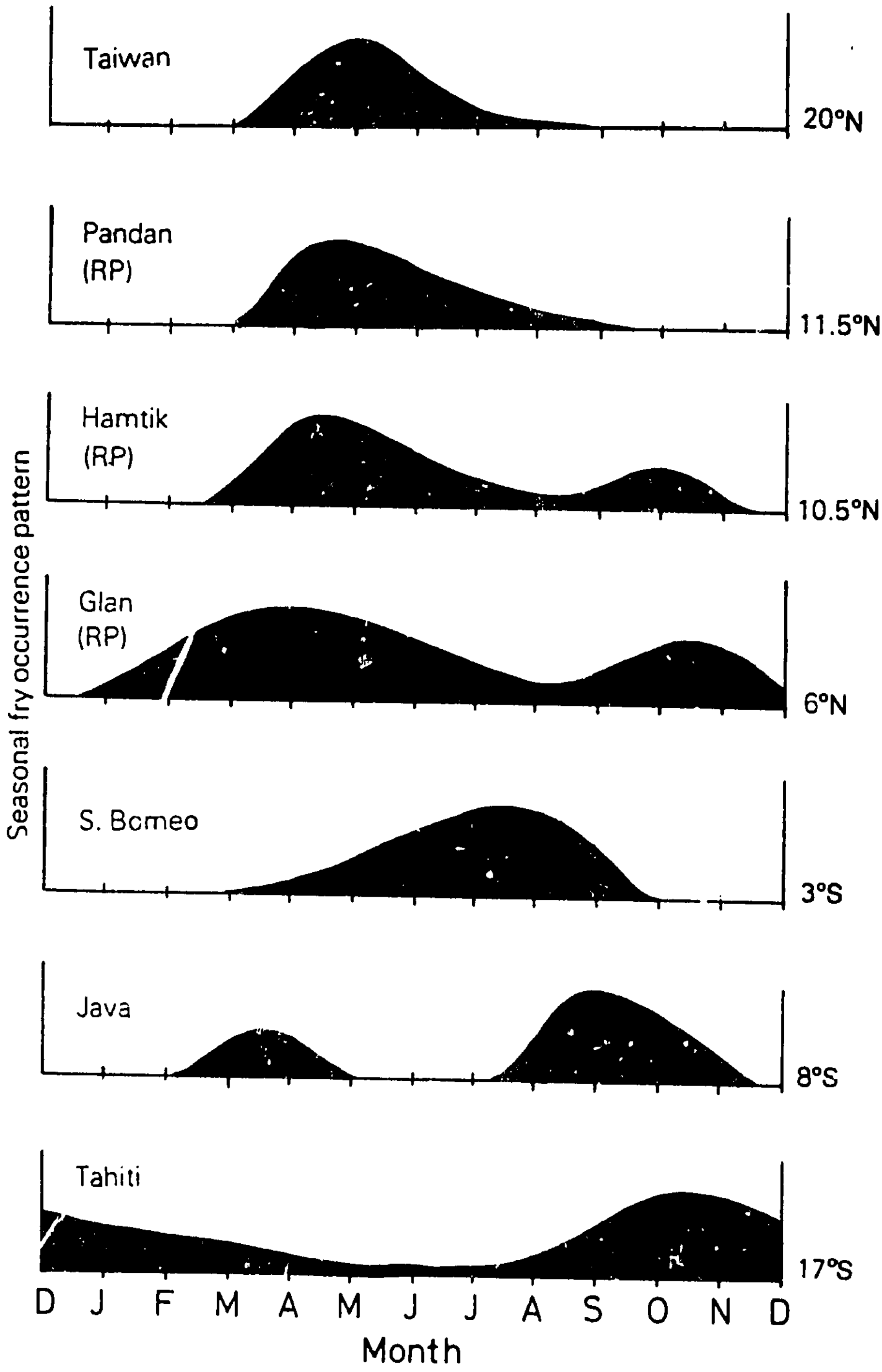

Fig. 1. Seasonal fry occurrence patterns at different latitudes. From Kumagai (1984).

1-5.3. POSSIBLE FACTORS INFLUENCING DISTRIBUTION

The factors influencing where and when wild animals occur, and how abundantly, are classic subjects in the fields of biogeography, ecological physiology, behavior and ecology. Natural situations almost universally involve many factors, with both short-term and long-term temporal components. These complexities are usually almost inextricably intertwined, with a wide range of complex interactions.

The distribution and occurrence of the milkfish is a classic example of one of these situations. Many authors have tried to sort out the varied lines of evidence. Schuster (1960) and Buri et al. (1981) have tried to synthesize both field and laboratory experimental evidence, and have generated a series of hypotheses which attempts to account for major features of the phenomena described in Sections 1-5.1 and 1-5.2. Some of their suggestions (with additions by the present authors) are:

1) The outer limits of the northern and southern distributions of the milkfish are probably set by minimum winter temperatures. Adult fishes become sluggish, and, in overwintering pond situations, more susceptible to diseases and parasite infections, at water temperatures below about 15^{o}C (see Section 8-4, 9-2). In nature, it is impossible to determine if low temperature operates directly on the fish (e.g. reducing metabolism to levels too low to sustain life) or indirectly (e.g. less active fish are more vulnerable to predation; lower temperatures are associated with changed or reduced food supplies; etc.).

2) The location and timing of spawnings by milkfish are almost certainly determined by complex interrelationships between local, short-term factors and long-term, more ultimate factors. Short-term factors probably include the status of energy reserves in the bodies of prospective spawners, phases of the moon, tidal and wind-generated current direc-

tions and velocities, water temperatures and, in higher latitude regions, day length. More ultimate factors probably include: distributions, abundances and varieties of planktonic foods needed by fry; distributions, abundances and varieties of potential predators on eggs, larvae and fry; proximity of suitable littoral aggregation areas for fry and of suitable inshore habitats for juveniles; relative absence of major predators on adult fish.

3) The great fecundity of female fish, which can produce in a spawning season from 1-9 million eggs each (depending on age, size, and condition of the fish; see Chapters 3 and 4), is presumably an evolutionary response to the great uncertainties and high embryonic, larval, postlarval and juvenile mortalities of the life history described above.

4) The wide tolerances for temperature (eurythermality), salinity (euryhalinity), and dissolved oxygen levels shown by the juveniles, subadults and adults are evolutionary bases for the ability of the species to occupy coastal and freshwater environments that subject the fish to a variety of severe environmental stresses.

5) Given the life history of the species (especially the apparently short duration of larval and fry stages), it seems probable that both the huge geographic distribution overall and its occurrence around many isolated island groups across the Pacific are mostly due to long distance movements of subadults and adult fish, rather than planktonic drifts of eggs, larvae and fry.

1-6. LIFE HISTORY, HABITATS, POPULATION DYNAMICS

The preceding sections have outlined the most important features of the life history stages of the milkfish and habitats occupied by each stage as they are known for the western Pacific. We present here a schematic diagram (Figure 2) summarizing this information graphically. We do not know

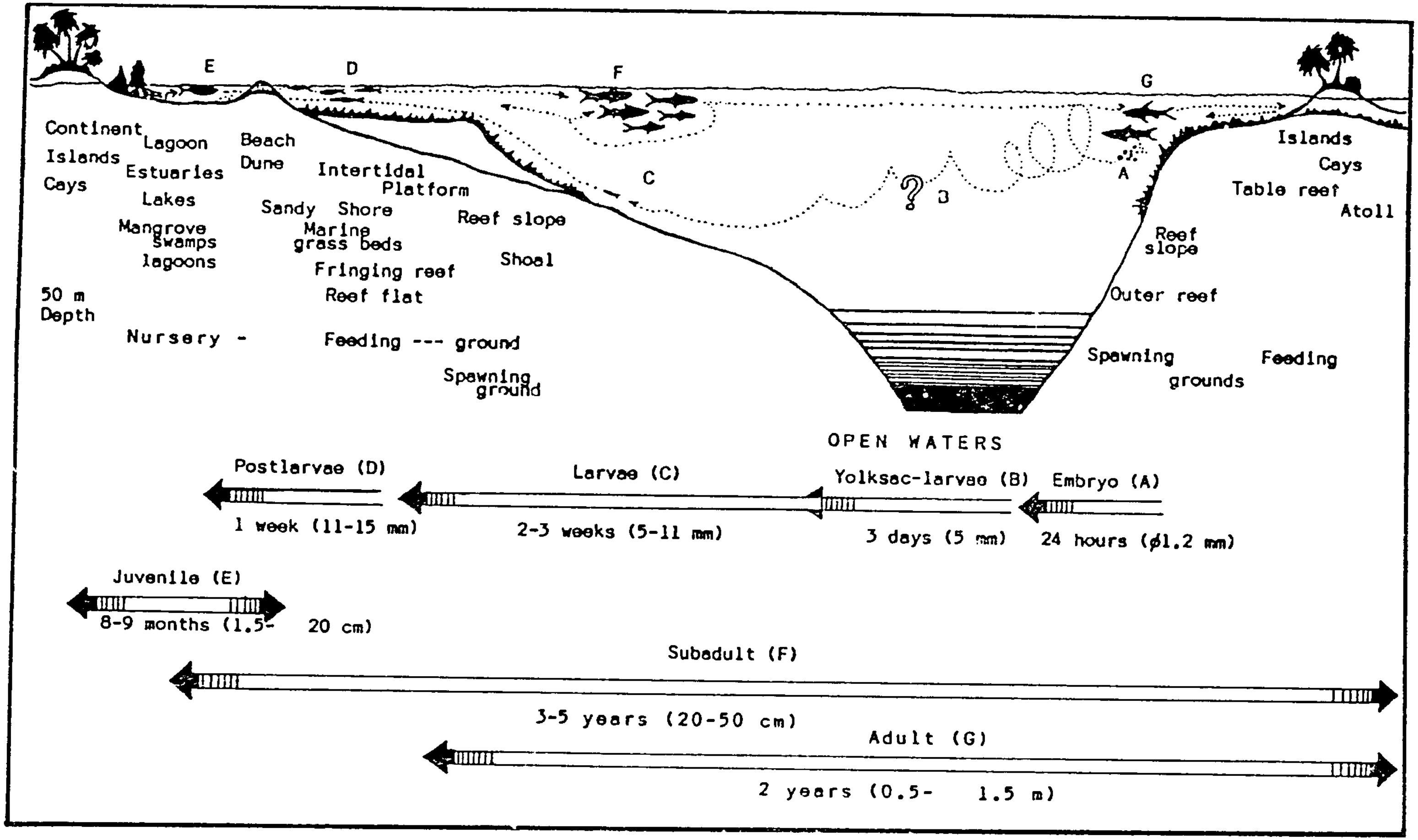

Fig. 2. Schematic diagram of the complete life history of milkfish with indications of major habitats and duration of each developmental stage. From Buri et al. (1981).

if milkfish living in environments radically different from the tropical western Pacific (e.g. the Red Sea, the Gulf of California) are the same.

Writing these last few sections has made the authors of this chapter aware of an anomaly in the milkfish literature as compared with the literature on virtually all other species of commercially-exploited finfishes. This anomaly is that there are no mathematically based papers on dynamics of milkfish populations in the wild (SEAFDEC, 1983).

There seem to be at least two primary reasons for this: 1) Fry are the growth stage that is commercially harvested, not subadult to adult fish. Most of the standard fisheries biological methods for studying the dynamics of exploited populations are designed for use with species exploited as adults. 2) The traditional industry obtains its supplies of fry mostly from large numbers of individual fisherpeople, few of whom are used to keeping records and many of whom operate in areas remote from trained fisheries personnel.

The absence of such literature may be important. Even if captive propagation of milkfish succeeds as well as it is likely to, understanding the dynamics of wild milkfish populations will be of practical value. There are several reasons for this. The industry uses many billion of fry each year. It is probable that, for many years, most of this supply will continue to be wild caught. Artificially-spawned fry are likely to be less available and more costly than wild ones, at least during peak spawning periods. It is also likely that it will be a long time before a network of hatcheries is developed that adequately covers most areas where milkfish are cultured.

1-7. NUTRITION

Chapter 7 provides a detailed description of most important aspects of milkfish nutrition, including summaries of what is known about wild fish from the fry to adult stages

(Sections 7-4.1, 7-4.2). In addition, Benitez (1984) carefully reviewed much of the same subject matter, reaching basically similar conclusions to those stated in Chapter 7, though with some differences in emphasis. There is no reason to repeat these materials here, so this section is restricted to 1) summarizing the most generally important highlights of these other discussions; and 2) pointing out several important limitations on present knowledge.

1) Important Highlights:

a) Milkfish, in all life history stages from fry onward, are primarily diurnally active, visually oriented, microphagous planktivores (meaning that they eat mostly small, whole planktonic organisms that they find by sight during daytime). Most of the individual items they eat are so small that they do not select among them for those that might be most nutritious. They capture most of their food by filtering it from water they take into their mouths, using their very long, dense arrays of gill rakers.

b) In addition to plankton collected in the water column, milkfish in all life history stages also feed on benthic detritus. Both animal and plant materials are eaten, with larger fish in the ocean seeming to prefer planktonic diatoms. Diatoms are important food items for all growth stages.

c) Juvenile milkfish in the wild that feed primarily on plant materials develop relatively longer intestines than juveniles feeding primarily on animal materials.

2) Limitations on present knowledge:

Work on milkfish nutrition is limited in both scope and variety. With respect to wild fish, studies have been geographically restricted, with the majority of work done in the central Philippines. Two exceptions are the papers by Chacko (1945) and Tampi (1958), who worked in India.

Excepting only fry, essentially nothing of an experimental nature has been done using wild fish. Nothing is known of feeding rates, digestive efficiencies, overall energy budgets, etc. in nature. It seems at least possible that more and better studies on wild fish could lead to some useful insights into culture methods and conditions.

Energy budgets seem especially necessary. There is no better approach known than such budgets in evaluation of the tradeoffs made by animals in their partitioning of metabolic energy into maintenance, growth, activity and excretion.

1-8. PHYSIOLOGY

Milkfish physiology is a neglected subject. This is unfortunate as the species has several unusual abilities clearly based upon its physiological capacities.

Section 1-5.1 pointed out that the species is euryhaline. Actually is extraordinarily so, at least in the juvenile to adult stages of its life. These stages seek out waters of salinities ranging from full-strength sea water (35-40 ppt salinity) down to fresh water (salinities below 0.5 ppt). In ponds, especially landlocked ponds in areas often having little rain (such as ponds on Christmas Island in the central Pacific: Crear, 1980), these growth stages survive in salinities above 100 ppt (Section 7-4.1; Benitez, 1984).

Experiments with fry (see Section 5-5, 6-6 to 6-8, 8-2) and observations of cultured juvenile milkfish in pens and ponds (see Section 8-1) show that the species is also eurythermal (approximate seasonal tolerance limits (10-40^{o}C) and tolerant of low dissolved oxygen (down to about 20% of air saturation) and high dissolved carbon dioxide concentrations. Milkfish survival in ponds with restricted exchanges of water, high biological oxygen demands, long-term exposures to full tropical sun in summer, high stocking densities, and low water temperatures in winter (e.g. in Taiwan) indicates that the fish have strong tolerances for, and resistances to, fairly extreme values of many other physicochemical variables as well.

A few experimental studies have been published which have attempted to quantify aspects of the physiological resistances of the species (particularly with respect to salinity, temperature, and dissolved oxygen concentrations) and to determine aerobic metabolic rates under different sets of environmental conditions. These studies have been based on wild-caught fry of different sizes and, in a few papers, smaller juveniles (the latter often obtained from culture ponds, not the wild). The experiments reported have also, virtually without exception, been structured so as to provide information relevant to captive maintenance or transport of the fish -- not to understanding the physiology of the species in relation to its natural environments. The papers involved are listed in the bibliographies of Chapters 5, 6 and 8, and on pages 22-24 of the SEAFDEC bibliography (SEAFDEC, 198?).

The result of this situation is that, with a limited exception for one aspect, the ecological physiology of the wild milkfish is unknown. Thus, there is no factual basis upon which it is possible to discuss either basic biological questions (such as the extent to which physiological limitations or capacities determine where and when the different growth stages of the species occur -- as compared with ecological limitations of other kinds) or practical questions (such as the extent to which different handling procedures or culture conditions are or are not physiologically optimal). There would appear to be substantial room for significant improvement of culture methods on the basis of suitable physiological studies.

The limited exception to the generalization made in the last paragraph relates to the developmental anatomy of locomotor musculature and of major sensory systems and aspects of the behavioral physiology of fry and juveniles in relation to their sensory development (results summarized in Section 5-3 and Kawamura, 1984). Major conclusions are: 1) fry of the sizes captured by the traditional industry are fully functional in their abilities to detect and respond to optical, chemical and mechanical stimuli; 2) active fry can maintain sustained swimming speeds of 9-11 cm/s; 3) young fry are strongly positively phototactic (they are attracted to visible light), while older fry approaching metamorphosis are only weakly so; 4) juveniles shortly after metamorphosis are photonegative, become photopositive about 2 months later, then behaviorally neutral to light after 2 more months; 5) fry orient strongly to water movements; and 6) fry show moderately developed and juveniles well developed optomotor reactions to movements of objects in their visual fields. (The ability to respond to, usually to follow, such movements is believed by some to be an important part of the basis for schooling behavior in fishes.)

These behavioral physiological results help account for several features of the field behavior of fry and juveniles. They have contributed to developments in the design of new types of collecting gear for fry (see Chapter 6).

There have also been a few papers published on aspects of milkfish tissue composition, tissue metabolism and biochemical activities. A list of most of these may be found on pp. 17-18 of the SEAFDEC bibliography (SEAFDEC, 1983). Gordon (1972) published results of measurements of muscle tissue metabolic rates in relation to temperature.

1-9. BEHAVIOR

Sections 1-5 through 1-8 in this chapter, also Section 3-5, 4-4, 5-3, 6-10, 7-3, 7-4, and 8-2 all include information on various aspects of behavior of milkfish in different growth stages and different situations. There is no reason to repeat these materials here. Comments here will be limited to a few major points and general observations.

1) Milkfish behavior is unstudied from a basic behavioral point of view. What is known about the species has been learned in the course of observations made for other reasons, primarily on fish from culture and primarily to assist the industry. Here again, as with physiology, there is a significant chance that basic behavioral studies on fish from the wild would contribute in important ways to improvement of culture methods.

2) Milkfish behavior has not been recognized as a significant subject for study, even in the applied context. There is, for example, no listing of behavior as a subject area in the SEAFDEC bibliography (SEAFDEC, 1983).

3) The complex life history, the abundance, the vast geographic distribution, the known relatively short-distance migrations and the unknown, but probable, long-distance movements of the species make it an excellent subject for future basic and applied behavioral studies in many respects. The long-distance movements raise important fundamental questions such as what are the stimuli for them, what mechanisms influence their frequencies, durations and directions, how do the fish navigate (if they do), etc. Comparably important sets of basic questions might also be approached through longitudinal studies of behavioral differences between major growth stages.

1-10. REPRODUCTION

As was noted in the Preface, the extensive and intensive efforts made by many people in many places in recent years to learn enough about milkfish reproduction to permit reliable, controlled captive spawnings was the primary stimulus for the production of this book. Chapters 3 and 4 summarize the current state of these (increasingly successful) efforts. Lam (1984), Liao and Chen (1984), and

Lee and Liao (1985) provide other recent reviews. There are more than 60 citations on reproduction listed in the SEAFDEC bibliography (pp. 99-105; SEAFDEC, 1983). Comment here is restricted to a few observations.

1) The long-standing difficulties people have had learning how to induce captive reproduction in this species seem partly to have been consequences of the unusual nature of the traditional industry, which in turn has been based upon the unusual life history of the fish. There has been little reason for milkfish farmers to keep adult fish in their ponds for the 5-7 years required for sexual maturation.

2) The conditions of life in most traditional milkfish ponds are so dramatically different from the natural environments in which adult milkfish spawn normally that those few mature adults in captivity have largely been deprived of the sensory stimuli and local circumstances that would usually trigger spontaneous spawning. Thus, it has required the advent of sophisticated knowledge of fish reproductive endocrinology to permit routine overcoming of the stress barriers to successful spawning.

3) It seems possible that the provision of more nearly natural pond culture conditions, plus properly fed fish of suitable ages, will lead before long to the development of reliable supplies of ripe fish that will spawn regularly and spontaneously in captivity, without need for hormonal stimulation. The possible role of external chemical communication (pheromones) in spawning should be studied.

4) The great fecundity of adults should permit a relatively small number of strategically located small breeding facilities to provide a significant fraction of the fry that the marketplace can handle, especially at time of year other than peak natural spawning periods. The wild population can be used as a continuing reservoir of new genetic material, to prevent undesirable inbreeding.

1-11. UTILIZATION OF MILKFISH

The traditional milkfish industry is described in detail in Chapters 5 through 10. The major product is fish for human food, usually in the form of fresh whole animals removed from their ponds or pens fewer than twelve hours before sale in retail markets (Taiwan, Philippines, Indonesia).

There are also a variety of other forms in which milkfish are marketed. Live fingerlings are increasingly used as baitfish by commercial open sea fishing operations fishing for other species, such as tuna (Ling, 1977; Lee, 1984). In both Taiwan and the Philippines, some market-size fish are chilled or frozen for export to a variety of countries overseas. An increasing number are also processed and canned for export overseas. Finally, a tiny proportion of the crop is sold as dried fish. Samson (1984) presents some data on tonnages and economic values for these various products, specifically for the Philippines.

1-12. SUMMARY

This chapter summarizes present knowledge of the biology of wild milkfish in their natural environments. It also includes some miscellaneous information not covered elsewhere in the book.

Though the milkfish has been farmed for at least 700 years, it has been known to science only since 1775. Over the years since, many different scientific names have been applied to the species, now called Chanos chanos (Forskal).

The external appearance of juvenile to adult fish is described; other chapters describe embryos and fry. Morphological variation is slight over the wide geographic distribution of the species. Two unusual anatomical features of the alimentary tract are the pharyngeal organs and the esophageal spiral valve.

The monotypic milkfish family was probably evolutionarily derived from a salmon-like ancestor. Closest evolutionary relationships are to either the carp-like fishes or the characins.

Milkfish occur throughout the tropical Indo-Pacific region. The life history starts with broadcast spawning of large numbers of eggs in the open sea. Spawning seasons vary regionally. After two to three weeks, surviving fry suddenly appear in large numbers in clear, shallow waters close inshore, usually off sandy beaches. These fry are actively collected by humans and are the basis for the traditional industry. After a few days the fry return to sea. One to two weeks later, and after metamorphosis into juveniles, they enter coastal estuaries, lagoons and swamps. Some go into freshwater streams and lakes. After extended stays in these environments, juveniles become subadults and return to the open sea. Sexual maturity is reached after five to six years. Adults also live in the ocean and probably carry out long distance movements. Major factors influencing milkfish distributions in space and time are discussed.

Little is known about the population dynamics, nutrition, physiology, behavior and reproduction of wild milkfish. From the fry stage onward, milkfish feed primarily on both plankton (especially algae) and detritus. They are tolerant of wide ranges of environmental temperature, salinity and dissolved oxygen and carbon dioxide concentrations. As sensory systems develop in fry, their behavior changes in ways adaptive to field conditions. Milkfish in captivity appear to be under environmental stresses that prevent their reproducing spontaneously.

In addition to their direct use as fresh food, milkfish are also frozen and canned, some are sold dried and some are used as bait for high seas fisheries for other species.

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REFERENCES

Benitez, L.V. 1984. Milkfish nutrition. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in milkfish biology and culture. Island Publishing House, Inc., Manila, Philippines. pp. 133-143.

Bridge, T.W. and G.A. Boulenger. 1910. In: S.F. Harmer and Z.E. Shipley (Eds.) The Cambridge Natural History, Vol. 3:1-312. London.

Buri, P., V. Banada and A. Trino. 1981. Developmental and ecological stages in the life history of milkfish, Chanos chanos Forsskal). Fish. Res. J. Philip. 6: 33-58.

Buri, P. and H. Motoh. 1980. The skull of milkfish, Chanos chanos (Forskal). Proc. Jap. Soc. Syst. Zool. 19: 45-52.

Chacko, P.I. 1945. On the food and alimentary canal of the milkfish, Chanos chanos Forskal. Curr. Sci. 14: 242-243.

Chandy, M. 1956. On the oesophagus of the milkfish Chanos chanos (Forsskal). J. Zool. Soc. India 8: 79-84.

Chandy, M. and M.G. George. 1960. Further studies on the alimentary tract of milkfish, Chanos, in relation to its food and feeding habits. Proc. Nat. Inst. Sci. India 26(B3): 126-134.

Chaudhuri, H., J. Juario, R. Samson and L. Tiro. 1975. Notes on the external sex characters of Chanos chanos (Forsskal) spawners. Fish. Res. J. Philip. 1: 76-80.

Chiu, Y.N. and L.V. Benitez. 1981. Studies on the carbohydrases in the digestive tract of the milkfish Chanos chanos. Mar. Biol. 6: 247-254.

Crear, D. 1980. Observations on the reproductive state of milkfish populations (Chanos chanos) from hypersaline ponds on Christmas Island (Pacific Ocean). Proc. World Maricul. Soc. 11: 548-556.

Fink, S.V. and W.L. Fink. 1981. Interrelationships of the ostariophysan fishes (Teleostei). Zool. J. Linnean Soc. 72: 297-353.

Fowler, H.W. 1959. Fishes of Fiji. Govt. of Fiji, Suva.

Gordon, M.S. 1972. Comparative studies on the metabolism of shallow-water and deep-sea marine fishes. I. White-muscle metabolism in shallow-water fishes. Marine Biol. 13: 222-237.

Gorriceta, I.R. 1982. Studies on the digestive lipases and lipid composition of milkfish, Chanos chanos (Forsskal). Unpubl. M.Sc. Thesis, Univ. Philip. System. 56 pp.

Greenwood, P.H., D.E. Rosen, S.H. Weitzman and G.S. Myers. 1966. Phyletic studies of teleostean fishes, with a provisional classification of living forms. Bull. Amer. Mus. Nat. Hist. 131: 339-456.

Herre, A.W. 1936. Fishes of the Crane Pacific expedition. Field Mus. Nat. Hist. Zool. Ser., Publ. 353: 27-29.

Herre, A.W. and J. Mendoza. 1929. Bangos culture in the Philippine Islands. Philip. J. Sci. 38: 451-509.

Johannes, R.E. 1981. Words of the lagoon. Univ. California Press, Berkeley, Calif.

Kapoor, B.G. 1954. The pharyngeal organ and its associated structures in the milkfish, Chanos chanos (Forsskal). J. Zool. Soc. India 6: 51-58.

Kapoor, B.G., H. Smit and I.A. Verighina. 1975. The alimentary canal and digestion in teleosts. Adv. Mar. Biol. 13: 109-239.

Kawamura, G. 1984. The sense organs and behavior of milkfish fry in relation to collection techniques. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in milkfish biology and culture. Island Publishing House, Inc., Manila, Philippines. pp. 69-84.

Kumagai, S. 1984. The ecological aspects of milkfish fry occurrence, particularly in the Philippines. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in milkfish biology and culture. Island Publishing House, Inc., Metro Manila, Philippines. pp. 53-68.

Kuwatani, Y. and T. Kafuku. 1978. Morphology and function of epibranchial organ studied and inferred on milkfish. Bull. Freshwat. Fish. Res. Lab., Tokyo 28: 221-236.

Lam, T.J. 1984. Artificial propagation of milkfish: present status and problems. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in milkfish biology and culture. Island Publishing House, Inc., Metro Manila, Philippines. pp. 21-39.

Lee, C.S. 1984. The milkfish industry in Taiwan. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in milkfish biology and culture. Island Publishing House, Inc., Metro Manila, Philippines. pp. 183-198.

Lee, C.S. and I.C. Liao (Eds.). 1985. Reproduction and culture of milkfish. Oceanic Institute, Hawaii and Tungkang Marine Lab., Taiwan.

Liao, I.C. and T.I. Chen. 1984. Gonadal development and induced breeding of captive milkfish in Taiwan. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in milkfish biology and culture. Island Publishing House, Inc., Metro Manila, Philippines. pp. 41-51.

Ling, S.-W. 1977. Aquaculture in southeast Asia: a historical overview. Univ. Washington Press, Seattle, Washington.

Moyle, P.B. and J.J. Cech, Jr. 1982. Fishes: an introduction to ichthyology. Prentice-Hall, Englewood Cliffs, New Jersey.

Nelson, G.J. 1967. Epibranchial organs in lower teleostean fishes. J. Zool. 153: 71-89.

Nelson, J.S. 1976. Fishes of the world. J. Wiley and Sons, New York.

Rabanal, H.R. and I.A. Ronquillo, 1975. Distribution and occurrence of milkfish, Chanos chanos (Forsskal). Proc. Natl. Bangus Symp. SEAFDEC: 20-33.

Rabor, D.S. 1938. Studies on the anatomy of the bangos, Chanos chanos (Forsskal). I. The skeletal system. Philip. J. Sci. 67: 351-377.

Ronquillo, I.A. 1975. Biological studies on bangos (Chanos chanos). Philip. J. Fish. 9: 18-37.

Rosen, D.E. and P.H. Greenwood. 1970. Origin of the Weberian apparatus and the relationships of the ostariophysan and gonorynchiform fishes. Amer. Museum Novitates 2428: 1-25.

Samson, E. 1983. The milkfish industry in the Philippines. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in milkfish biology and culture. Island Publishing House, Inc., Metro Manila, Philippines. pp. 215-228.

Schuster, W.H. 1960. Synopsis of biological data on milkfish Chanos chanos (Forsskal), 1775. FAO Fish. Biol. Synopsis No. 4: 1-64. Fish. Div., Biol. Branch, Food and Agriculture Org., United Nations.

SEAFDEC. 1983. Milkfish bibliography. Library, Aquaculture Department, SEAFDEC, Iloilo, Philippines.

Senta, T. and A. Hirai. 1980. Records of milkfish, Chanos chanos (Forsskal) from mainland Japan. Bull. Fac. Fish. Nagasaki Univ. 48: 13-18.

Senta, T. and S. Kumagai. 1977. Variation in the vertebral number of milkfish Chanos chanos, collected from various localities. Bull. Fac. Fish. Nagasaki Univ. 43: 35-40.

Tampi, P.R.S. 1958. On the food of Chanos chanos (Forsskal). Indian J. Fish. 5: 107-117.

Therezien, Y. 1976. Donnè sur Chanos chanos Forsskal 1775 (famille des Chanidae) à Madagascar. Bull. Cent. Etude Rech. Sci. Biarritz 11: 35-52.

Winans, G.A. 1980. Geographic variation in the milkfish Chanos chanos. I. Biochemical evidence. Evolution 34: 558-574.

Winans, G.A. 1985. Geographic variation in the milkfish Chanos chanos. II. Multivariate morphological evidence. Copeia (4): 890-898.

2. POPULATION STRUCTURE

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2-1. INTRODUCTION

The milkfish, Chanos chanos, has a very broad geographic distribution, extending from the Indian Ocean to the west coast of Mexico and Central America (Villaluz et al., 1983). Such a broad geographic range undoubtedly exposes this species to a variety of habitats. It also creates geographic, environmental and temporal barriers to gene flow. This extensive distribution raises some obvious questions about the genetic uniformity of milkfish. Information on the population structure of this species is academically interesting because it can clarify the origin of new species and the processes that bring about within-species differentiation (i.e., establishment of discrete populations). The need for this information is highlighted by the scarcity of published reports describing the population structure of tropical marine fishes in the Pacific (Fujino, 1976; Erlich, 1978; Winans, 1980; Bell et al., 1982; Shaklee, 1983 and 1984).

The identification of discrete stocks (as defined by Ihssen et al., 1981) of milkfish can serve several very important practical purposes as well, among them avoiding the current plight of the salmonid fishes. Many species of the salmonid group are now extinct or on the verge of extinction mainly because of man's activities (i.e., hatchery practices, overharvesting, habitat destruction, etc.) (Ros, 1981; Christensen and Larsson, 1979; Johansson, 1981). Although some of these problems do not apply to the milkfish, the great interest in culturing this species suggests we should examine problems stemming from inappropriate management policies or indiscriminate hatchery operations.

In the last decade, many salmonids have been shown to exist as discrete stocks (Child, 1977; Allendorf and Utter, 1979; Ryman et al., 1979; Altukov, 1981; Ferguson and Mason, 1981; Kornfield et al., 1981; Ryman, 1981; Ryman and Stahl, 1981). Also, strains and subspecies of fishes have been reported to exhibit differences in many physiological, behavioral and ecological attributes. For example, within-species groups have been shown to differ in such important characteristics as growth rate and time of maturation (Beamish and Tsuyuki, 1971; Moav et al., 1974); time of river entry (Berg, 1959); growth and fecundity (Ricker, 1972; Loftus, 1976); sensitivity to temperature (Bulger and Schultz, 1979); time of spawning (Averett and Espinosa, 1968); age of smoltification (Refstie et al., 1977); swimming stamina (Green, 1964); pH tolerance (Robinson et al., 1976; Swarts et al., 1978); migratory behavior (Raleigh, 1967; Bams, 1976) and others (see Ihssen et al., 1981).

An example of an inappropriate management policy is one that assumes a single panmictic group when more than one such group exists. Although this assumption simplifies implementing of the management policy, it results in favoring isolated stocks over others, and does not benefit the entire fishery as intended. Management policy decisions must be made with a thorough understanding of the structure and dynamics of the system. (Gall, 1972; Allendorf and Utter, 1979; Maclean and Evans, 1981; Shaklee, 1983).

Overfishing one of the salmonids, the Atlantic salmon, Salmo salar, led to the release of massive numbers of hatchery-reared fish to compensate for the decline in naturally-produced fish. It is estimated that as many as 60% of the smolts occurring in the Baltic Sea are artificially produced (Christensen and Larsson, 1979; Johansson, 1981). This large dependence on hatchery-produced fish and the practice of using a small number of individuals for broodstock has leached genetic variability from this species and from other hatchery-produced fishes (Allendorf and Utter, 1979; Ryman and Stahl, 1980; Allendorf and Phelps, 1981). The loss of genetic diversity is very dangerous because it limits a species' adaptation to changing environments on a large geographic scale. Individual variation within local populations is also diminished (Odum, 1971; Smith and Chesser, 1981). Lack of genetic diversity makes a species vulnerable to extinction which negates the intent of supplementing natural stocks with hatchery fish.

Although the amount of milkfish fry currently produced via induced maturation and spawning makes a negligible contribution to the total milkfish fishery, with further improvement in the artificial propagation of this species (Kuo et al., 1979; Liao et al., 1979; Kuo et al., 1979; Juario et al., 1984) hatchery practices need to be carefully designed to circumvent the problems experienced with the salmonids.

The fisheries manager is faced with the formidable task of developing effective management policies that will preserve the diversity found in natural populations. In contrast, a fish culturist selects for different morphological and physiological traits within a species in order to exploit those characteristics that are economically beneficial in production cultures (Moav, 1979). Thus, fish managers and culturists appear at first to be interested in opposite areas of fish biology. One tries to conserve the natural diversity of the fish stocks; the other tries to shape natural variation into traits with economic potential. Both fish culturists and managers, however, share common interests: to discover population subdivisions of the species in question and to estimate the amount of genetic diversity within the species.

The importance of these common interests is reflected in the tremendous increase in published reports and symposia on measuring, managing, and exploiting the genetic diversity of aquatic organisms (Loftus and Regier, 1972; Simon and Larkin, 1972; Colby, 1977; Hjelm, 1981; Maclean and Evans, 1981; Wilkins and Gosling, 1983).

2-2. METHODS

Numerous characteristics and techniques can be employed for stock identification. Ihssen et al. (1981) presents a review of the advantages and shortcomings of the many available techniques.

Electrophoretic analysis of protein variation is the method of choice for estimating genetic diversity and population structure within species and proposed systematic relationships among species (Avise, 1974; Allendorf and Utter, 1979; Shaklee, 1983; Buth, 1984). The basis of the electrophoretic technique is a simple one. Tissue homogenates are placed in porous, homogeneous matrix (i.e. cellulose acetate, polyacrylamide, starch gel, etc.), and the gel is then subjected to an electric current. The soluble proteins present in the homogenate will migrate differentially (direction and rate) depending on the net charge of the proteins. After a suitable duration, the electric current is removed and the gel is stained according to any of a variety of histochemical procedures. The kinds of proteins visible are usually enzymes because their unique catalytic nature identifies them as bands of activity on the gel. With experience, the bands of activity can be interpreted as a biochemical phenotype. Because of the linear relationship between the nucleotide sequence of the DNA molecule and the amino acid sequence of the protein, the differential migration of a polypeptide can be interpreted as representing a particular genotype (Fig. 1).

To determine the stock structure of a particular species, the investigator must first determine the amount of genetic variation that is electrophoretically detectable. The frequency distribution of the detected allelic variants is then catalogued for samples (i.e., populations) throughout the species' range. Lastly, the observed variation at the gene loci is then subjected to statistical analysis with the null hypothesis being that the samples are being drawn from a single panmictic group. Significant differences in the frequency distribution of variant alleles at a single polymorphic locus would suggest that stocks are discrete (Fig. 2). This interpretation would be strengthened if the observed differences exhibit consistent patterns when many polymorphic loci are included.

2-3. STOCKS IN THE HAWAIIAN ISLANDS

The existence of a heterogeneous population structure of milkfish was first reported utilizing the cellulose acetate electrophoretic technique on eye lens proteins (Smith, 1978). Smith reported that milkfish caught in Hawaiian waters could be classified into three categories. One sample of individuals caught in Pearl Harbor on the island of Oahu was characterized by one banding pattern. Another sample collected on the island of Hawaii, some 320 km distant, consisted of individuals with protein banding patterns that fell into three categories. In addition, the majority of individuals that were caught near the island of Hawaii had banding patterns not seen in the Oahu sample. Such a result strongly

Glucose Phosphate Isomerase Phenotypes Of Chanos chanos

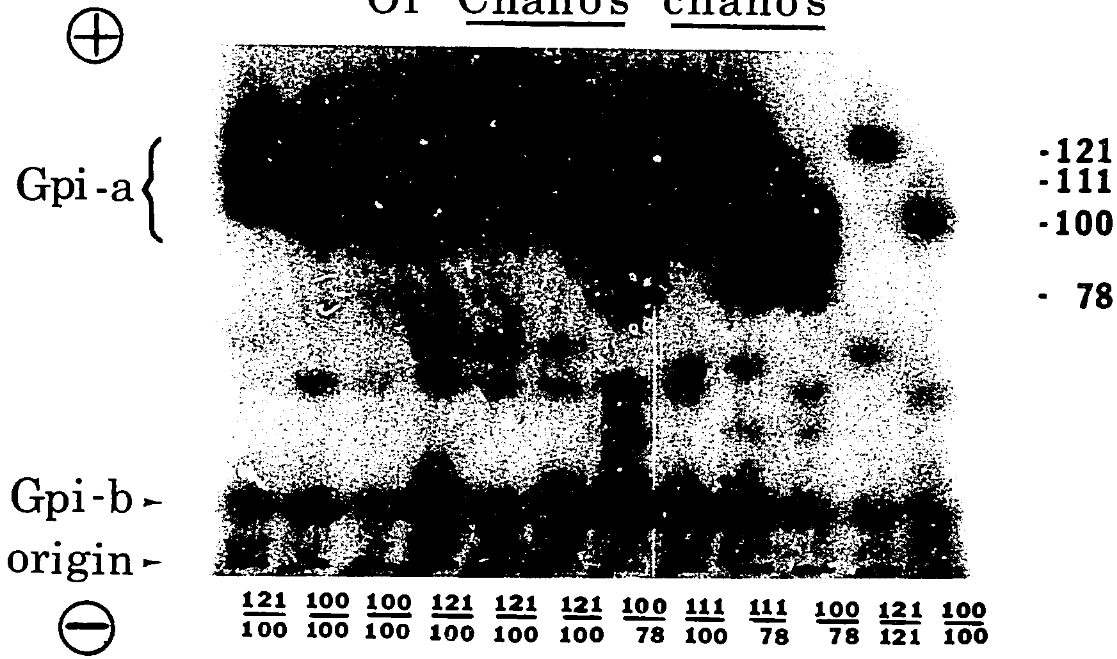

Fig. 1. Glucose phosphate isomerase (GPI) phenotypes of Chanos chanos.

A zymogram depicting the phenotypes of GPI isozymes from a random sample of twelve milkfish fry collected from Hawaii Kai, on the island of Oahu, Hawaii. In summary, the GPI molecule is a dimer consisting of 2 subunits to form a functional enzyme molecule. Identical subunits as found in a homozygous state result in a single band of activity. The presence of two varying subunits being codominantly expressed in the heterozygous state is clearly identified by the 3 banded phenotype. The variety of phenotypes presented may be interpreted as resulting from the presence of 4 alleles at the presumed GPI-A locus. The proposed genotype for each individual is provided at the bottom of the figure. The assignment of the alleles is based from those of Winans (1980).

Phosphogluconate Dehydrogenase Phenotypes

From Wild and Cultured Milkfish

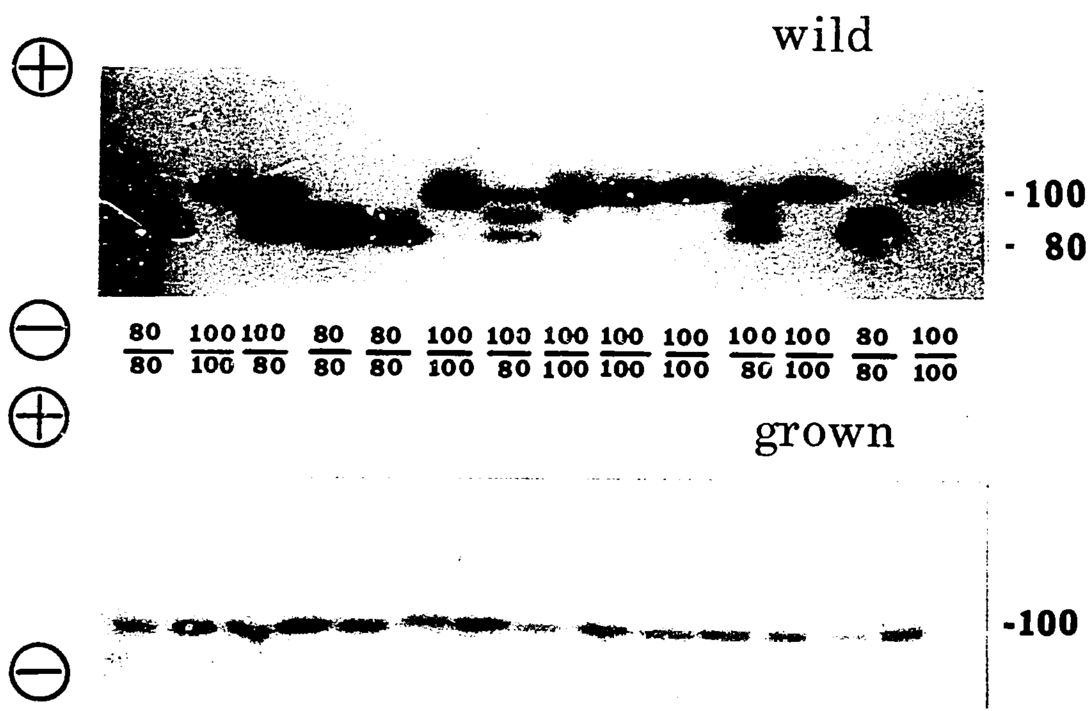

Fig. 2. Phosphogluconate dehydrogenase (PGDH) phenotypes from wild and cultured milkfish populations. Two electrophoretically detectable alleles are found at the presumed PGDH locus from milkfish individuals collected at Hawaii Kai, on the island of Oahu. The allelic frequencies are dramatically shifted as seen in the zymogram of PGDH from offspring that resulted from induced spawning trials held at the Oceanic Institute, Hawaii. The large shift in allelic frequency is the result of matings between a limited number of parents.

suggests a discreteness of stocks between the two island groups. A major criticism of this study is that the structural and genetic basis of the eye lens proteins is currently unknown (see Shaklee, 1984).

2.4. POPULATION STRUCTURE IN THE PACIFIC

A larger study investigating several specific questions on the genetic diversity and population structure of milkfish in the Pacific was conducted with the starch-gel electrophoretic procedure (Winans, 1980). The specific questions were: (1) What is the level of genetic variation in this species? (2) What is the population structure of the fish in the Pacific? and (3) How genetically different are Hawaiian milkfish populations relative to other groups? To answer these questions, approximately 50 individuals from 14 locations throughout the Pacific were collected, frozen and shipped to Hawaii for analysis (Table 1). Each individual was screened for 20 enzymes and protein systems representing the gene products for 38 gene loci.

The number of electrophoretically-detectable alleles was then catalogued for each location and can be summarized by different values. The estimates of the genetic diversity of milkfish resulting from the analysis of 38 gene loci are:

Average number of alleles per locus - 1.48 ± 0.08

Proportion of polymorphic loci - $P_{.99} = 0.233 \pm 0.06$

Average heterozygosity per locus - $H = 0.075 \pm 0.011$

One in four loci is polymorphic. In comparison with average heterozygosity values of other fish on which data are available, milkfish have apparently higher levels of genetic variation. The interpretation of these results, however, requires a great deal more experimenting and testing (Nevo, 1978; Winans, 1980).

The electrophoretic data were analyzed in three different ways to determine the population structure of milkfish in the Pacific. The first procedure utilized all 38

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Table 1. Localities of Milkfish Samples

Location	Abbreviation	Groupings
San Thomas, Luzon	P8	Philippine Islands
Mercedes, Luzon	P7	
Hamtique, Panay	P6	
Ormoc, Leyte	P5	
Argao, Cebu	P4	
Cagayon de Oro, Mindanao	P3	
Zamboanga, Mindanao	P2	
Sulop, Mindanao	P1	
Palau	PAL	Equatorial Pacific Islands
Tarawa	TAR	
Fanning Island	FAN	
Christmas Island	CHR	
Hawaii	HAW	Hawaiian Islands
Oahu	OAH	

****

gene loci simultaneously. Such a procedure involves summarizing the data into an estimate of genetic relatedness among the groups sampled. This estimate is the distance coefficient of Nei (1978). The average genetic distance observed for all locations was 0.002. This value is very low when compared to values obtained for other organisms of continental origin. The distance value of milkfish, however, is about average when compared with available values of other marine fishes (Winans, 1980).

To visualize the relationships among the groups analyzed using the distance coefficient, a dendrogram was constructed from a clustering analysis (Sokal and Rohlf, 1963). In summary, four major groups emerge from the cluster analysis. All of the samples from the Philippines constitute one group. Palau, Tarawa, Fanning Island and Christmas

Island constitute another group. The third group is the Hawaiian Islands. Lastly, the islands of Oahu and Hawaii are themselves discrete from each other (Fig. 3).

The second means of determining stock structure involved grouping samples according to the highest possibility of gene flow. This was followed by a locus-by-locus analysis of the six most polymorphic loci observed. From this analysis, three somewhat different patterns of population differentiation emerged. Two loci indicated a pattern in which the Philippine Islands and the equatorial Pacific Islands were grouped together while the islands of Oahu and Hawaii were represented by two distinct groups. Two other loci indicated a second pattern: the Philippines as one group, the equatorial Pacific Islands as a second group and the Hawaiian Islands as a third group. In this case, however, no difference was found between the islands of Oahu and Hawaii. The last pattern observed for the final two loci indicated four groups: 1) the Philippines, 2) the equatorial Pacific Islands, 3) the island of Oahu, and 4) the island of Hawaii. This final distribution pattern resembles the one that emerged with the clustering analysis of the genetic distance coefficients.

The last method employed to investigate stock structure of milkfish was to test for clinal or latitudinal variation. Four polymorphic loci had statistically significant regressions of gene frequency on latitude. However, in light of the other results (i.e., distance analysis and the locus-by-locus analysis) Winans (1980) has interpreted the observed clinal variation as the result of comparing three genetically independent groups.

The highlight of this study was the consistent demonstration of the distinctiveness of the Hawaiian Island milkfish population from those elsewhere in the Pacific. The low genetic distance values suggest, however, that all

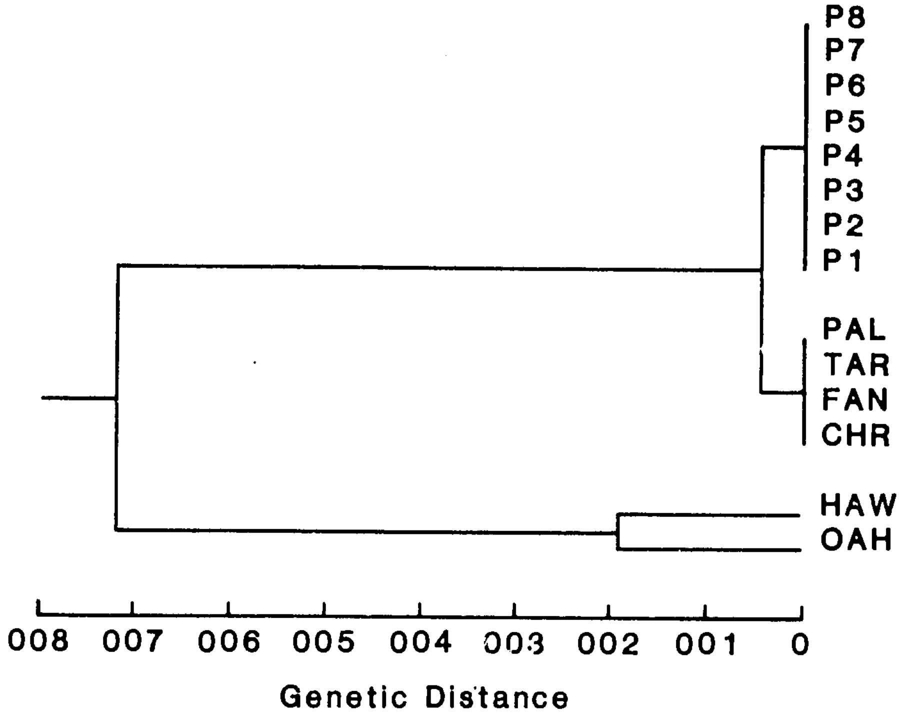

Fig. 3. Dendrogram of Nei's Genetic Distance Coefficient.

The clustering analysis reveals three or possibly four populations of milkfish in the Pacific. They are: 1) Philippines Islands, 2) Equatorial Pacific Islands, and 3) Hawaiian Islands. The fourth possible group is between the island of Oahu and Hawaii, within the Hawaiian Islands Group (from Winans, 1980).

milkfish groups tested are very similar genetically. One might conclude that a high degree of population intermixing must be taking place in this species throughout the Pacific. An obvious question would be how widespread is the phenomenon among other fishes or other marine organisms in the Pacific? Such information may give insight into the degree to which oceanographic parameters influence the gene flow of these organisms.

Several questions on the population structure of *Chanos chanos* have arisen since Winan's 1980 work. The discreteness of stocks between the islands of Oahu and Hawaii is surprising. Milkfish from these two locations have a large genetic distance relative to the other localities studied, since these two Hawaiian islands are separated by only 20 km. This dilemma is highlighted by another electrophoretic analysis of two other fish species, *Stegastes fasciolatus* and *Pristipomoides microlepis*, throughout the Hawaiian chain. No differentiation of samples taken from the entire length of the Hawaiian Archipelego (some 2500 km) was found (Shaklee, 1983; Shaklee and Samallow, 1984). *S. fasciolatus* is territorial as an adult and lays demersal eggs which would lead one to predict that, if there were to be any kind of stock differentiation, it would have been found in this species and not *C. chanos* or *P. microlepis*, which both produce hydrated eggs that float.

The high degree of similarity found within the Philippine Island group is contradicted by reports of two seasonal peaks in abundance of milkfish fry. One peak occurs around April in southern Mindanao, another peak occurs in April and June around the middle portion of the Philippine Islands (Villaluz et al., 1983). This suggests that there are different milkfish stocks in the Philippines, and not the uniform group reported in Winan. In his study, the majority of samples collected from the different localities in the

Philippines were obtained from fish ponds. The origin of these milkfish is questionable given the methods by which they were stocked into fish ponds. Obtaining and selling milkfish fry for stocking into ponds is a thriving business in the Philippines (Villaluz et al., 1983). The journey from collector to concessionaire to pond owner would undoubtedly mix the fry sufficiently that one could easily envision the genetic uniformity reported in his study.

2.5. SUMMARY

The need to analyze the genetic diversity and population structure of milkfish and any species that is being considered for domestication was discussed. In summary, there are both academic as well as practical reasons for such investigations to be conducted.

The state of the art method for estimating the degree of genetic diversity and population structure is starch-gel electrophoresis. Protein variation in milkfish as revealed by electrophoretic techniques was presented as an example of the type of data used in defining stock structure.

A study utilizing protein electrophoresis indicates at least three and possibly four intraspecific groups, depending upon the interpretation of results and the statistical analysis employed. One consistent pattern that does emerge is the discreteness of the Philippine Islands from the equatorial Pacific Islands, versus the Hawaiian Islands. Further evidence suggests an additional subdivision within the Hawaiian Islands themselves.

Future work should focus on the Philippine Islands because of questions regarding the sampling scheme used in the above study. In addition, a discreteness of stocks is suggested by the seasonal and geographic variation in milkfish fry abundance. Likewise, studies conducted on other organisms in the Hawaiian Islands report genetic uniformity, a finding which conflicts with past work suggesting a discreteness in milkfish stocks. Future work should focus on a re-evaluation of these stocks.

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REFERENCES

Allendorf, F.W. and F.M. Utter. 1979. Population genetics. In: W.J. Hoar, D.J. Randall and J.R. Brett (Eds.) Fish Physiology. Academic Press. New York. pp. 407-454.

Allendorf, F.W. and S. Phelps. 1981. Use of allelic frequencies to describe population structure. Can. J. Fish. Aquat. Sci. 38: 1507-1514.

Altukhov, Y.P. 1981. The stock concept from the viewpoint of population genetics. Can. J. Fish Aquat. Sci. 38: 1523-1538.

Averett, R.C. and F.A. Espinosa. 1968. Site selection and time of spawning by two groups of Kokanee in Odell Lake Oregon. J. Wildlife Manag. 32: 76-81.

Avise, J.C. 1974. Systematic value of electrophoretic data. Syst. Zool. 23: 465-481.

Bams, R.A. 1976. Survival and propensity for homing as affected by presence or absence of locally adapted paternal genes in two transplanted populations of pink salmon (Oncorhynchus gorpuscha). J. Fish. Res. Board Can. 33: 2716-2725.

Beamish, R.J. and A. Tsuyuki. 1971. A biochemical and cytological study of the longnose sucker Catostomus catostomus and large and dwarf forms of the white sucker Catostomus commersoni. J. Fish Res. Board of Can. 28: 1745-1748.

Bell, L.J., J.T. Moyer and K. Numachi. 1982. Morphological and genetic variation in Japanese populations of the anemonefish _Amphiprion clarkii_. Mar. Biol. 77: 99-108.

Berg, L.S. 1959. Vernal and hiemal races among anadromous fishes. J. Fish. Res. Board Can. 16: 515-537.

Bulger, A.J. and R.S. Schultz. 1979. Heterosis and interclonal variation in thermal tolerance in unisexual fishes. Evolution. 33: 848-859.

Buth, D.G. 1984. The application of electrophoretic data in systematic studies. Ann. Rev. Ecol. Syst. 1984. 15: 501-522.

Child, H.R. 1977. Biochemical polymorphism in char (_Salvelinus spinus_ L.) from Llynnau, Peris, Lwellyn and Bodlyn. Heredity. 38: 359-365.

Christensen, O. and P.O. Larsson. 1979. Review of Baltic salmon research. ICES Coop. Res. Rep. 1979(89). 124pp.

Colby, P.J. 1977. Introduction to the proceedings of the 1976 Percid International Symposium (PERCIS). J. Fish Res. Board Can. 34: 1890-1899.

Erlich, P.R. 1978. The population of coral reef fishes. Ann. Rev. Ecol. Syst. 6: 211-247.

Ferguson, A. and F.M. Mason. 1981. Allozyme evidence for reproductively isolated sympatric populations of brown trout _Salmo trutta_ L. in Lough Melvin, Ireland. J. Fish Biol. 18: 629-642

Fujino, K. 1976. Subpopulation identification of skipjack tuna specimens from the southwestern Pacific Ocean. Bull. Jap. Soc. Sci. Fish. 42: 1229-1235.

Gall, G.A.E. 1972. Phenotypic and genetic components of body size and spawning performance. In: R.W. Moorehead (Ed.) Progress in Fishery and Food Science. Univ. of Washington Publications in Fisheries. New Series, Vol.5. Seattle, Washington. pp. 159-163.

Green, P.M. Jr. 1964. A comparison of stamina of brook trout from wild and domestic parents. Trans. Am. Fish. Soc. 93: 96-99.

Hjelm, L. 1981. Opening Address. In: N. Ryman (Ed.). Fish gene pools. Ecol. Bull. (Stockholm) 34: 11-12.

Ihssen, P.E., H.E. Booke, J.M. Casselman, J.M. Mcglade, N.R. Payne and F.M. Utter. 1981. Stock identification: material and methods. Can. J. Fish Aquat. Sci. 38: 1838-1855.

Johansson, N. 1981. General problems in Atlantic salmon rearing in Sweden. In: N. Ryman (Ed.). Fish gene pools. Ecol. Bull. (Stockholm), 34: 75-83.

Juario, J.V., M.N. Duray, V.M. Duray, J.F. Nacario and J.M.E. Almendras. 1984. Induced breeding and larval rearing experiments with milkfish Chanos chanos (Forsskal) in the Philippines. Aquaculture, 36: 61-70.

Kornfield, I., K.F. Beland, J.R. Moring and F. Kireis. 1981. Genetic similarity among endemic arctic char (Salvinus alpinus) and implications for their management. Can. J. Fish Aquat. Sci. 38: 32-39.

Kuo, C.M., C.E. Nash and W.O. Watanabe. 1979. Induced breeding experiments with milkfish Chanos chanos (Forsskal) in Hawaii. Aquaculture. 18: 95-105.

Liao, I.C., J.V. Juario, S. Kumagai, H. Nakajima, M. Natividad and P. Buri. 1979. On the induced spawning and larval rearing of milkfish Chanos chanos Forsskal. Aquaculture, 18: 75-93.

Loftus, K.H. 1976. Science for Canada's fisheries rehabilitation needs. J. Fish Res. Board Can. 33: 1822-1857.

Loftus, K.H. and H.A. Regier. 1972. Introduction to the proceedings of the 1971 symposium on salmonid communities in oligotrophic lakes. J. Fish. Res. Board Can. 29: 613-616.

Maclean, J.A. and D.O. Evans. 1981. The stock concept, discreteness of fish stocks and fisheries management. Can. J. Aquat. Sci. 38: 1889-1898.

Moav, R. 1979. Genetic improvement in aquaculture industry. In: T.V. R. Pillay and W.A. Dill (Eds.) Advances in Aquaculture. Fishing News Books Ltd. Farnham, Surrey. 653 pp.

Moav, R., G. Hulata and G. Wohlfarth. 1974. The breeding potential of growth curve differences between the European and the Chinese races of the common carp. 1st World Congress on Genetics Applied to Livestock Production. 3: 573-578.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Nevo, E. 1978. Genetic variation in natural populations: patterns and theories. Theoretical Population Biology, 13: 121-177.

Odum, E.P. 1971. Fundamentals of ecology. 3rd ed. W.B. Saunders Co., Philadelphia, Pennsylvania. 546 pp.

Raleigh, R.F. 1967. Genetic control in the lakeward migration of sockeye salmon, Oncorhynchus nerkii fry. J. Fish Res. Board Can. 24: 2613-2622.

Refstie, T., T.A. Steine and T. Gjedrem. 1977. Selection experiments with salmon. II. Proportion of Atlantic salmon smoltifying at one year of age. Aquaculture. 10: 231-242.

Ricker, W.E. 1972. Heredity and environmental factors affecting certain salmonid populations. In: R.C. Simon and P.A. Larkin (Eds.). The stock concept in Pacific Salmon. H.R. Macmillan lectures in Fisheries. Univ. British Columbia. Vancouver B.C. pp. 19-160.

Robinson, G.D., W.A. Dunson, J.E. Wright and G.E. Mamolito. 1976. Differences in low pH tolerance among strains of brook trout (Salvelinus fontinalis). J. Fish. Biol. 8: 5-17.

Ros, T. 1981. Salmonids in the Lake Vanern area. In: N. Ryman (Ed.) Fish gene pools. Ecol. Bull. (Stockholm) 34: 21-31.

Ryman, N. 1981. Conservation of genetic resources: Experiences from the brown trout (Salmo trutta). In: N. Ryman (Ed.). Fish gene pools. Ecol. Bull. (Stockholm). 34: 61-74.

Ryman, N. and G. Stahl. 1981. Genetic perspectives of identification and conservation of Scandinavian stocks of fish. Can. J. Fish Aquat. Sci. 38: 1562-1575.

Ryman, N., F.W. Allendorf and G. Stahl. 1979. Reproductive isolation with little genetic divergence in sympatric populations of brown trout (Salmo trutta). Genetics, 92: 247-262.

Shaklee, J.B. 1983. The utilization of isozymes as gene markers in fisheries management and conservation. In: Isozymes: Current Topics in Biological and Medicinal Research (11): 213-247.

Shaklee, J.B. 1984. Genetic variation and population structure in the damselfish, Stegastes fasciolatus throughout the Hawaiian Archipelago. Copeia. 1984(3): 629-640.

Shaklee, J.B. and P.B. Samollow. 1984. Genetic variation in a deepwater snapper, Pristipomoides filamentosus, in the Hawaiian Archipelago. Fishery Bull., Vol.82, No.4, pp. 703-713.

Simon, R.C. and P.A. Larkin (Eds.). 1972. The Stock Concept in Pacific Salmon. H.R. Macmillan Lectures in Fisheries. Univ. of British Columbia, Vancouver. B.C. pp. 19-160.

Smith, A.C. 1978. Pathology and biochemical genetic variation in the milkfish, Chanos chanos. J. Fish. Biol. 13: 173-177.

Smith, M.H. and R.K. Chesser. 1981. Rationale for conserving genetic variation of fish gene pools. In: N. Ryman (Ed.). Fish gene pools. Ecol Bull. (Stockholm) 34: 13-20.

Sokal, R.R. and F. S. Rohlf. 1969. Biometry. W.H. Freeman and Co., San Francisco. 775 pp.

Swarts, F.A., W.A. Dunson and J.E. Wright. 1978. Genetic and environmental factors involved in increased resistance of brook trout to sulfuric acid solutions and mine acid polluted water. Trans Am. Fish. Soc. 107: 651-677.

Villaluz, A.C., W.R. Villaver, and R.J. Salde. 1983. Milkfish fry and fingerling industry of the Philippines: Methods and practices. Technical Report #9, 2nd edition. Aquaculture Department, SEAFDEC. International Development Research Center. 81 pp.

Wilkins, N.P. and E.M. Gosling. 1983. Genetics in aquaculture. Elsevier Science Publishers. B.V. New York. 426 pp.

Winans, G.A. 1980. Geographic variation in the milkfish, Chanos chanos. I. Biochemical Evidence. Evolution. 34: 558-574.

3. REPRODUCTION

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3-1. INTRODUCTION

Milkfish (Chanos chanos) have been cultured for centuries, particularly in Southeast Asia. The only source of seed for milkfish farming is the ocean and catches are unreliable. Unlike many other cultured fish, the spawning of milkfish in captivity has not been routinely successful. The current status of milkfish spawning is discussed in detail in Chapter 4. The major constraint to developing an induced spawning technique for milkfish is the lack of available mature fish. Optimal conditions required for maturation, spawning and reproduction of milkfish are poorly understood.

In this chapter, available information on the reproduction of milkfish will be reviewed and discussed. Complete understanding of milkfish reproduction is the key to achieving control of breeding in this species.

3-2. SEXUALITY

The milkfish is a heterosexual fish. Hermaphroditism in this fish has not been reported.

3-2.1. SEX DETERMINATION

Research on milkfish reproduction is complicated by the apparent lack of sexual dimorphism in this species. A convenient method to determine the sex of living milkfish is still under investigation.

The sex of milkfish can be determined histologically when the fish are at least two years old (Liao and Chen, 1983), but this requires the biopsy of the fish gonad and so does not meet the needs of fish farmers. Fish must survive sex determination in order for maturation to be induced.

Chaudhuri et al. (1976) described morphological differences in the anal region of mature male and female milkfish. The male has two main openings visible externally, the anterior anus and the posterior urogenital opening at the tip of the urogenital papilla. The female was observed to have three openings, the anterior anus, followed by the genital pore and the urinary pore located posterior to the genital pore at the tip of the urogenital papilla. Liao and Chen (1979) found these differences to be invalid for smaller, tank-reared virgin fish. Perhaps the Chaudhuri technique may only be valid in mature fish during the spawning season.

External features such as coloration, shape of the head, snout and operculum, presence of tubercles or nasal pores, and length, size, shape or roughness of the various fins were examined for use as sex determinants without success by Chaudhuri et al. (1976).

Smith (1978) indicated that the coelomic fluid of the sea urchin, Echinometra oblonga, could be used to determine sex in milkfish. He found that the red blood cells of female milkfish reacted with the coelomic fluid of the sea urchin, causing the blood to agglutinate. This reaction either did not occur with blood from males or the reaction was very weak and the results were inconsistent. Further evaluation is required before this technique can be applied by both researchers and farmers.

In light of this problem, several methods by which sex has been determined in other organisms have been examined at the Oceanic Institute for possible use on milkfish. They are: karyotyping; protein electrophoresis of serum proteins; quantification of vitellogenin; quantification of circulating levels of steroids, testosterone and estradiol-17β; and cannulation. Chromosome spreads of milkfish have been obtained (Arai et al., 1976); however, the spreads were obtained from only two juvenile specimens and did not exhibit any morphological differences. In addition, the chromosomes of milkfish, as with most other fishes, are extremely small and thus difficult to examine. The usefulness of this approach has not yet been fully determined. The effort involved in the production of usable milkfish karyotypes appears to make this approach impractical.

The slab polyacrylamide electrophoretic technique was used to determine if qualitative differences in serum proteins existed between the sexes of milkfish. To date, the blood serum proteins have not provided any markers useful in the determination of sex in milkfish.

Vitellogenin, a precursor molecule for yolk, can be measured in the blood of certain fishes utilizing radioimmunoassay procedures. The assay used to measure vitellogenin in milkfish serum employs an antibody directed against salmon vitellogenin. The antibody against salmon vitellogenin shows little cross-reactivity with the milkfish's vitellogenin molecule. As expected, when using an antibody with a low affinity for the desired molecule, the assay used showed a very poor ability to distinguish between males and females.

The circulating levels of the steroids testosterone and estradiol-17β found in milkfish serum were examined by radioimmunoassay procedures. The testosterone levels analyzed from the blood of milkfish individuals of known sex demonstrated that testosterone is not a good discriminator of sex in milkfish. Estradiol-17 β, however, does show some promise as a sex marker for female milkfish. All individuals that had a value of 300 picograms and above per milliliter of blood were correctly identified as females. Overall, of 38 individuals analyzed, an 82% success rate of sex determination was achieved.

Most researchers rely on the examination of gametes obtained by aspiration via polyethylene cannulae for determination of sex and state of maturation. The application of this technique is not difficult when the fish possesses mature gonads. However, experience is required for sexing fish whose gonads are at an earlier maturational stage, and the technique is impractical for determining the sex of immature fish.

3-2.2 SEX RATIO

There are no reports on the sex ratio of milkfish in natural populations. A sample of pond fish was comprised of 68 males and 84 females (Schuster, 1960). Kuo and Nash (1979) sampled milkfish from Hawaiian waters during a period of 36 months, collecting 104 males and 135 females. Recently, sampling of wild milkfish in Hawaii produced 69 males and 71 females (Lee et al., 1986b). In the above cases, the number of females is slightly higher. These results suggest, however, that the sex ratio of milkfish is nearly equal. These data represent the ratio of sexually matured fish of 4 years or older. Juario (see Lam, 1986) indicated that the spawning groups caught in fish traps in the Philippines were usually composed of one female with three or four males. However, sex ratios in the ponds where natural spawning occurred were one to one (Lin, 1985) and were one to two or three in induced spawning groups (Kelley et al., unpublished). Sex ratios of spawning groups in other areas were unknown. This information will be important to the attempt to achieve natural spawning in captivity.

3-2.3. THE GONADS

Testes -- A pair of gonads is located on the dorsal side of the intestines, attached to the peritoneal wall by mesochium. The testes extend anteriorly all the way to the liver and have very long genital ducts, posteriorly. Both genital ducts unite to form a common duct just before the urogenital pore. The opening of the urogenital pore is located posterior to the anal pore. Spermatozoa, or fully developed sperms, are produced in elongated and enlarged testes of mature males during the spawning season. Milt oozes from the urogenital pore when pressure is applied to the abdomen of a fully mature male. As with other finfish, the earlier developing stages of male gametes are called spermatogonia, spermatocytes and spermatids. The developing stages can be determined under regular light microscopy.

Ovary -- A pair of ovaries is located at the dorsal side of the intestines and is suspended from the dorsal peritoneal wall by mesovariam. The ovary is of "cystovarian" type, as in most teleost fishes, but has a very thin connective tissue capsule. Immature fish possess very long oviducts. The length of the ovary increases toward the genital pore as the fish matures. The genital pore is separate from and anterior to the urinary pore (Chaudhuri et al., 1976). The anus is located anterior to the genital pore.

The spawned eggs are pelagic in normal seawater. The average diameter is around 1.2 mm and varies slightly among geographic locations, races and individuals. There are no oil globules present in the eggs. The yolk is finely segmented (Delsman, 1929) and characterized as yellowish and frothy by Chacko (1950). Eggs become transparent after being spawned and fertilized. Unfertilized eggs remain opaque.

There are no reports on the relationship between the size of milkfish and its fecundity, chiefly because of limited sources of mature females from the sea. The limited data of Liao and Chen (1979) and Schuster (1960), indicate a relationship between total length and fecundity (Fig. 1). Fecundity increases as fish size increases and varies from less than one million eggs to as many as seven million (Schuster, 1960).

3-3. ENDOCRINOLOGY

3-3.1. PITUITARY

Tampi (1953) first described the pituitary gland of milkfish fry and fingerlings. Olsson (1974) described the fine structure of the pituitary in larval milkfish. He found that the orohypophysial duct is retained until metamorphosis, and concluded that morphological prerequisites exist in the milkfish for direct action of the environment on the prolactin-secreting cells. No mention was made of other pituitary cells. Tan (1985) conducted a comprehensive study on milkfish pituitaries at various maturational stages. Several unique features of the milkfish pituitary, as compared to other fish species, were also discussed in this report.

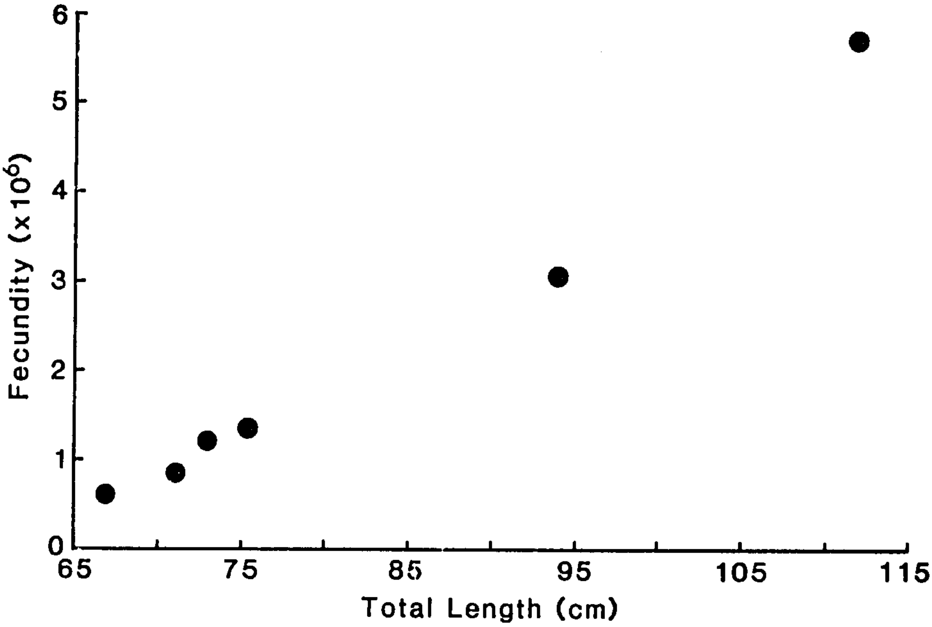

Fig. 1. Relation between total length and fecundity in milkfish. Data from Liao and Chen (1979) and Schuster (1960).

The pituitary, a small spherical body, is located midventrallis between the optic lobes and just behind the optic chiasma. It is enclosed in a bony chamber, is attached to the brain by the infundibular stalk, and consists of the dorsal and ventral lobes. Instead of extending dorsoventrally or antero-posteriorly, it extends anteriorly from its point of attachment at the base of the brain. The pituitary exhibits no sexual dimorphism in either gross morphological or histological aspects. The size of the pituitary increases with maturity and reaches the maximum size in fish with mature gonads. In fish with spent gonads, however, the pituitary remains the same size as in fish with mature gonads. Periodic acid schiff-positive [PAS (+)] basophils are found in all regions of the adenohypophysis instead of in the proximal pars distalis alone or one of the other regions, as in other teleosts. The PAS (+) basophils of the proximal pars distalis apparently increased in cytoplasmic staining intensity, degree of granulation and hypertrophy during sexual maturation and showed shrinkage and degranulation in fish with spent gonads. They are assumed to be gonadotrops and involved in the hormonal control of sexual maturation and spawning. The PAS (+) basophils in the rostral pars distalis are confirmed to be thyrotrops by Nagahama (Tan, 1985).

Descriptions of the milkfish pituitary can also be found in Tampi (1951).

3-3.2. GONADOTROPIN

Radioimmunological assay (RIA) methods for measuring the gonadotropin (GtH) level in milkfish have not yet been developed. The serum GtH levels in adult milkfish were measured by the salmon-GtH-RIA system and ranged from 3.4 to 8.8 ng/ml during the month of September in the Philippines (Marte and Crim, 1983). From the dose-response curves for standard salmon GtH and milkfish GtH, Marte and Crim (1983) concluded that the molecules of both GtHs are immunologically distinct. An RIA for measuring milkfish GtH needs to be developed.

Sherwood et al. (1984) indicated that the milkfish brain contains a peptide chromatographically and immunologically identical to synthetic salmon gonadotropin-releasing hormone (Gn-RH). Gn-RH was found to be present in both immature 7-month-old and 4-year-old milkfish, with the older fish containing more Gn-RH than the younger.

3-3.3. SEX STEROIDS

Sex steroids in milkfish serum, such as estradiol-17 β and testosterone have been measured by RIA (Marte and Lam, 1985). Mean estradiol and testosterone levels in females at different stages of maturity are as follows: immature - 267.5 ng/ml and 2.5 ng/ml; maturing - 2.8 ng/ml and >10 ng/ml; mature - 7.1 ng/ml and 33.8 ng/ml; spent - 1.4 ng/ml and 1.9 ng/ml. In males, testosterone levels increase from 4.9 ng/ml in immature fish to 31.5 ng/ml in mature fish. Preliminary data indicate the presence of seasonal and diurnal fluctuations in serum steroid levels. Fluctuation of steroid levels in the same individual at different developmental stages will be determined by ongoing experiments at the Oceanic Institute in Hawaii. A thorough understanding of changes in serum steroid levels is expected in the near future.

3-4. MATURATION

3-4.1. GONAD DEVELOPMENT

Gonadal development of milkfish is classified into several stages from macroscopic appearance, histological changes and gonadosomatic index (GSI = $\frac{\text{gonad weight}}{\text{body weight}}$ X 100). Liao and Chen (1983) staged the development of gonads of both male and female milkfish from their macroscopic appearance and histological changes: immature virgin, developing virgin, maturing, mature, gravid, spawning and spent (Table 1).

In males, the testes are small, thread-like, elongate

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Table 1. Stage of gonadal development in milkfish by different researchers.

Liao and Chen (1983)	Tan (1982)
Immature virgin Developing virgin	Immature
Maturing	Maturing
Mature Gravid and Spawning	Mature
Spent	Spent

****

structures which are pinkish-grey in color and found in fish at or before the developing virgin stage. Testes size increases with gonadal development. In mature males, the testes are rosy-white and occupy about 2/3 of the peritoneal cavity. From a histological point of view, spermatids and spermatozoa are not present in the male fish until they reach the maturing stage.

In immature virgin females, the macroscopic appearance of the gonad is the same as in males. In developing virgin females, discernible ovigenous lamellae are present, and, at the maturing stage, ovarian oocytes become visible to the naked eye. The color of female gonads changes from reddish-orange to yellowish-orange or bright yellow. Eggs are opaque right after they are spent. Histologically, the oocytes from fish in the developing virgin stage are at perinucleolus stage. Some primary yolk globule oocytes are present in the ovaries of fish at the maturing stage. At maturity, the majority of oocytes are at yolk globule stage.

Using GSI and histological appearance, Tan (1982) classified the development of milkfish, using GSI and histological appearance, into immature fish, maturing fish, mature fish and spent fish (Table 1). The GSI of males varied from

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Table 2. Gonadosomatic Index ($GSI = \frac{\text{gonad weight}}{\text{body weight}} \times 100$) of milkfish at different stages of maturation (data from Tan, 1982)

Maturational Stage	Male	Female
Immature	0.015 ~ 0.05	0.02 - 0.49 (0.2 mm)*
Maturing	0.09 ~ 0.76	0.23 - 1.30 (0.24 - 0.55 mm)*
Mature	0.32 ~ 3.05	1.84 - 7.62 (0.5 - 0.93 mm)*

* egg diameter

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0.015 to 3.05 and fluctuated between 0.02 to 7.62 in females (Table 2). Higher GSI values have been reported in many other locations. Crear (1980) reported that mature females on Christmas Island had GSIs ranging from 15 to 25. Mature female fish caught in Taiwan waters possessed a GSI of 24.91 (Liao, 1971). Liao and Chen (1979) recorded GSIs of 8.1 to 11.2 in females injected with hormones. In Hawaii, female milkfish had GSIs ranging from 7.07 to 9.76 during the spawning season (Kuo and Nash, 1979). The GSIs for males are lower than that of females. The GSI of mature males was reported between 3.1 and 5.4 in Hawaii (Kuo and Nash, 1979). The difference in GSIs among various reports can result from either genetic variation or stage of maturity. A conclusion cannot be drawn from available information.

Histologically, Tan (1982) staged ovarian maturation according to Yamazaki (1965) and testicular maturity after Funk and Donaldson (1972). At the immature stage, female milkfish have oocytes in the perinucleolus stage and the testes contain spermatogonia. Oocytes in maturing fish develop to the secondary yolk granule stage and testes show all stages of spermatogenesis. In mature fish, the predominant oocytes in the ovary are in the tertiary yolk granule stage. The seminiferous tubules of the testes of mature males are filled with spermatozoa. After spawning, ovaries contain empty follicles, atretic oocytes and oocytes in all stages of maturation. In spent males, seminiferous tubules shrink and contain few spermatogenic cells.

3-4.2. MATURATION AGE AND SIZE

The gonads of both male and female milkfish appear thread-like in fish less than 3 years old. Liao and Chen (1984) indicated there are only undifferentiated germ cells in the gonads of fish less than 2 years. Spermatogonia in males and previtellogenic oogonium and chromatin-nucleolus in females are found histologically in fish of 2 or more years of age. Liao and Chen (1983) reported that mature spermatozoa were first observed in a 4-year-old male. Running milt was observed in fish older than 5 years. Evidence suggests that females require a longer period to attain maturity than males. Vitellogenesis did not occur until fish were at least 5-years-old, but yolk globule stage eggs were present only in fish that were 6-years-old or older (Liao and Chen, 1983). Therefore, it was suggested that males older than 5 years and females older than 6 years show gonadal maturation in captivity. The maturation and natural spawning of 5-year-old fish, however, was achieved in floating cages located in a coastal area of the Philippines (Marte et al., 1983). The dimensions of the floating cages are 10 m in diameter and 3 m in depth. The fish are maintained under natural conditions. It appears milkfish may reach sexual maturity before the age of 6 if they are cultured under appropriate conditions.

The minimum size for the sexual maturation of milkfish varies among geographic locations. In the coastal waters of Taiwan, the smallest fish with mature gonads was 2.5 kg

(Liao, 1971). Mature fish from several hypersaline ponds (100 - 130 ppt) on Christmas Island had mean weights of only 1 kg (Crear, 1980). The maturation of milkfish as small as 2.65 kg has been induced by hormone manipulation in Hawaii (Lee et al., 1986b). The maturation of smaller fish was not attempted in this particular experiment. A conclusion has not yet been reached as to the importance of either age or size (see Lam, 1986).

3-4.3. CONDITIONAL FACTORS FOR MATURATION

Ambient conditions for promoting the onset of sexual maturation of milkfish are not yet known, although they have been inferred from many ecological studies (see review by Lee, 1985). Those assumptions need to be verified in controlled experiments.

1) Nutrition

Milkfish are generally known as herbivorous fish. Liao (1971) reported that gut contents of wild-caught adult milkfish were composed of crustaceans. Other observations indicate that milkfish consume animal food such as trash fish and krill (personal experience and communication).

Milkfish also ingested brine shrimp in a pond with 60 ppt salinity. An inspection of the digestive tract showed that brine shrimp accounted for 25% by volume of the diet (Crear, 1980). He stated that brine shrimp are the essential diet for sexual maturation of milkfish in hypersaline ponds on Christmas Island because mature milkfish were not found where brine shrimp were absent.

The protein content of diets used in maturation facilities ranges between 32 and 46%. Marte et al. (1984) and Lacanilao and Marte (1980) fed a diet containing 40% protein at 2.0% body weight to captive broodstock one year prior to expected spawning in floating cages. Lin (1984) obtained natural spawning in an earthen pond. The feed was composed of a mixture of rice grain, wheat flour, soybean meal and formulated eel feed offered at 5% total biomass once a day. Algae, yeast and vitamins E and B were given as supplements occasionally. Lee and Weber (1983) maintained milkfish broodstock in concrete tanks on catfish chow (32% protein) and obtained mature fish. Recently, milkfish broodstock fed on trout chow (39% protein) matured also (Lee et al., 1986a and b).

Therefore, a protein content of 32% in the diet can meet the nutritional requirements of broodstock.

2) Environmental Factors

Lee (1985) reviewed the correlation of environmental factors (light, temperature, salinity, space and stress) in the maturation of milkfish.

Light -- Kumagai et al. (1978) proposed that changes in light intensity stimulate gonadal maturation of milkfish based on the concurrence of the peak fry season with maximum intensity of sunlight in the Philippines. Lee and Weber (1983) succeeded in inducing maturation of milkfish in an indoor rectangular concrete block tank (5.2 X 4.8 X 1.3 m depth) with eight 6' fluorescent lights as the light source. They controlled the photoperiod regime at 6D:18L for three months, 12L:12D for the next three months and 18L:6D until the fish matured four months later. Milkfish also matured and spawned in an earthen pond where light intensity was depressed by a phytoplankton bloom (Lin, 1984). Spawning of milkfish in that pond occurred at night and during the day. Recent observation of milkfish spawning at Oceanic Institute also supports this finding; however, most spawnings occurred at night. In the wild, Delsman and Hardenberg (1934) reported that spawning took place in the evening from 8 to 10 pm.

It seems that intensity is not critical to the maturation and spawning of milkfish, although further study on photoperiod is necessary.

Temperature -- Wainwright (1982) indicated that the minimum temperature for spawning of milkfish is 24°C, based on fry seasonality. Fry season began with a rise in sea surface temperature and ended with its decline. He concluded that length of fry season was related to the mean annual sea surface temperature.

Studies on milkfish maturation have been conducted in warm water: 25-31°C in sea cages (Lacanilao and Marte, 1980), 21.4-30.7°C (Liao and Chen, 1979; Hsiao and Tseng, 1979) or 24-26°C in outdoor tanks (Lee et al., 1986a and b), and around 26°C in indoor tanks (Lee and Weber, 1983). Mature fish were found under all these conditions. No studies have been conducted to determine the minimum temperature for gonadal maturation of milkfish. A recent study (Lee et al., unpublished) found that milkfish which received luteinizing hormone-releasing hormone analogue (LHRH-a) cholesterol pellet implants matured in water temperatures of 20-22°C. These results suggest that milkfish can mature at water temperatures higher than 20°C.

Salinity -- The milkfish is a euryhaline species and matures in a wide range of salinities. Crear (1980) reported mature milkfish were found in a hypersaline pond (125ppt). In Hawaii, milkfish matured in lava-formed ponds at a salinity of 8-12ppt (Kuo, 1982). In the Philippines, mature milkfish were caught during their migration from Lake Naujan (freshwater) into the ocean (personal communication with SEAFDEC). However, spawning of milkfish in extreme salinities was not observed. Problems associated with inducing final maturation of milkfish in extreme salinities have been discussed (Kuo et al., 1979). Kuo et al. (1979) recommended that salinities in spawning tanks be gradually adjusted to a normal range for the successful induction of final maturation.

3-4.4. HORMONAL INDUCTION

Lacanilao et al. (1985) administered hormones alone or in various combinations by intramuscular (IM) injection, IM or intraperitoneal (IP) pellet implantation or IP infusion to immature fish (3.5 to 5.5 years old) or wild regressed milkfish. The hormones administered included acetone-dried salmon pituitary (SPH), partially purified salmon gonadotropin (SG-G100), L-thyroxine sodium (T4), estradiol-17β (E2), Durandron Forte (a long-acting testosterone preparation) and human chorionic gonadotropin (HCG). The results of these treatments were negative. Lacanilao et al. (1985) explained that many factors may have caused this lack of gonadal response. Factors include unsustained release of hormone from the cholesterol pellet, size of holding structures, unstable environmental conditions, inappropriate hormone dosage, inadequate methods or frequency of hormone administration and possible production of anti-hormone after prolonged treatments.

Lee et al. (1986d) attempted to induce spermatogenesis in immature milkfish by orally administering testosterone. The GSI values for the fish receiving 12.5 mg and 25 mg of testosterone per kg of body weight were consistently higher than that of the control group. Although spermatogenesis was prompted by testosterone treatments, the size of the testes remained very small. Fish in this experiment were under 2 kg and may have been too small to mature (see 4-4.2). Optimal dosage and condition of the fish (size and age) are important factors for positive results.

Implantation of cholesterol pellets containing exogenous hormones such as LHRH-a and silastic tubing packed with 17α-methyltestosterone (17-MT) providing timed release, has recently been used on adult milkfish in Hawaii, with very promising results (Lee et al., 1986a and b). Each pellet contained 200 µg LHRH-a and each capsule had 250 µg of 17-MT in oil form. After milkfish received the combination treatment of both pellet and capsule, the milkfish matured and began spawning about one month earlier than the normal spawning season.

3-5. SPAWNING

3-5.1. SPAWNING GROUNDS

Spawning grounds for milkfish were characterized by Schuster (1960) as clear, shallow waters with sandy or coal bottoms, at a distance of not more than 30 km from shore. In Philippines waters, Senta et al. (1980) recorded 26.7°C as the minimum temperature at locations with milkfish eggs in Philippine waters. Kuronuma and Yamashita (1962) reported 27°C was the determining temperature for the appearance and disappearance of milkfish fry in Vietnamese waters. Other environmental factors, such as current and wind, may also influence selection of spawning grounds by milkfish to insure the survival of eggs and larvae. Actual factors determining the spawning ground are still unknown.

3-5.2. SPAWNING SEASON

Various studies have been conducted to determine the natural spawning seasons of different milkfish populations Spawning seasons can be determined from information on gonadal histology, gonadosomatic index or the abundance of eggs and fry.

Histological examination of gonadal development is the most precise way to determine the spawning season. Staging gonadal development was described in Section 3-4.1.

The GSI fluctuates according to the maturity of fish and the spawning season and is therefor concurrent with the peak of the annual monthly GSI distribution. Tan (1982) reported the GSI ranges for immature, maturing and mature fish (Table 2). Mature male and female milkfish had GSIs greater than 0.32 and 1.84, respectively. Unfortunately, determining spawning season through histological examination or GSI fluctuation involves the sacrifice of valuable adult fish.

The spawning season can also be determined by the pattern of fry abundance. According to Liao et al., (1979), spawning takes place two weeks before fry season because fry reached the coast at the age of 10-15 days. The length of the fry collection season differs according to latitude (Kumagai, 1984). The spawning season at different geographical locations varies in length and time of year at which it occurs. Kumagai (1984) concluded that the fry season, in the northern hemisphere, is longer toward the equator and shorter at the higher latitudes.

Spawning season lasts from June to August in Hawaii (Kuo and Nash, 1979), late May through August in Taiwan (Liao and Chen, 1979), March through December with a peak in April and May in Thailand, Vietnam, the Philippines and Fiji (Schuster, 1960), and from March to June and again from September to December in Indonesia and India (Schuster, 1960). It seems that the spawning season begins earlier and lasts longer to the south and west of Hawaii.

In 1985, a group of fish received monthly hormone implantations from March to June. The fish were checked monthly from April to December. Most of the fish possessed tertiary yolk globule stage eggs every month and were used in induced spawning trials (Lee et al., 1986c). Therefore, milkfish are capable of multiple spawns and the spawning season can be extended.

3-5.3. SPAWNING BEHAVIOR AND MIGRATION

Many aspects of spawning behavior in milkfish are still not known. Based on the abundance of fry during the new and full moon periods, Kumagai et al. (1978) suggested that the spawning activity of milkfish occurs during the first and last quarter moons. Senta et al. (1980) concluded that wild milkfish spawn at night.

Lin (1984) detected chasing behavior two to three days before spawning in a pond. Time of spawning was frequently between midnight and 3 am, although daytime spawning also took place (Lin, 1984). Milkfish receiving hormone implantation spawned spontaneously at different times of day in tanks (Lee et al., 1986c): however, most of those which were fertilized occurred between midnight and 10:30 am.

Spawning behavior was recently recorded by Kelley et al. (unpublished). In all six fertilized spawns observed, the release of eggs by the females was stimulated by one, or more commonly, both of the two males present. The most frequent behavior exhibited by the males was vent-nuzzling, both on the female and on each other. In several cases, the males appeared to press the female's abdomen simultaneously from both sides which possibly facilitated egg release. The "cooperation" observed between the two males suggests that a sex ratio of two males to one female may be essential. Milkfish which possess developing ovaries were observed to migrate from Lake Naujan (freshwater) to the ocean during the prespawning months of November to March (SEAFDEC, pers. comm.). Spawning of milkfish in freshwater has not been observed. Therefore, this type of migration of milkfish may be classified as spawning migration.

3-6. SUMMARY

The information available on the reproduction of milkfish is sparse despite its importance to the control of propagation of this species. Furthermore, some information is contradictory. In order to reach accurate conclusions on the reproduction of milkfish, controlled experiments must be designed and carried out. A summary of the current available information follows.

Milkfish are a heterosexual species with a sex ratio in the wild of one to one. There is no sexual dimorphism in this species. Sex differentiation in gonads is not significant until the fish are 2 years old.

Knowledge of reproductive endocrinology is restricted to the structure of the pituitary and the levels of estradiol-17β and testosterone in blood. No standard method is available for measuring gonadotropin. The Gn-RH extracted from milkfish brains is identical to synthetic salmon Gn-RH.

The maturation of this species is not fully understood. The minimum maturational size and age in normal seawater is 2.5 kg and 5 years, respectively. These parameters change, however, with alterations in culture conditions. A long daylength, warm water temperatures (greater than 20°C), and a diet high in protein (32%) favor maturation. The recent development of a hormone implantation technique furthers the ability to control maturation.

The spawning season occurs at different times of the year and varies in length according to geographic location. Each individual female spawns several times during the season. Spawning usually occurs at night but has been observed during the day. Observations of spawning behavior indicate the need for two or more males per female for fertilized spawning to occur.

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REFERENCES

Arai, R., K. Nagaiwa and Y. Sawada. 1976. Chromosomes of Chanos chanos (Gonorynchiformes, Chanidae). Jap. J. Ichthyology, Vol. 22, No. 4. pp. 241-242.

Chacko, P.I. 1950. Marine plankton from waters around Krusadai Island. Proc. Indian Acad. Sci 31(3): 162-174.

Chaudhuri, H., J. Juario, R. Samson, and L. Tiro. 1976. Notes on the external sex characters of Chanos chanos (Forskal) spawners. Fish. Res. J. Philipp. 1(2): 76-80.

Crear, D. 1980. Observations on the reproductive state of milkfish populations (Chanos chanos) from hypersaline ponds on Christmas Island (Pacific Ocean). Proc. World Maricul. Soc. 11: 548-556.

Delsman, H.C. 1929. Fish eggs and larvae from the Java Sea. Treubia 9(2): 276-286.

Delsman, H.C. and J.D.F. Hardenberg. 1934. De indische zeevissen en zeevisserij. Visser and Co., Batavia, Indonesia. 388 pp.

Funk, J.D. and E.M. Donaldson. 1972. Induction of precocious sexual maturity in male pink salmon (Onchorhynchus gorbuscha). Can. J. Zool. 50: 1413-1419.

Hsiao, S.M. and L.C. Tseng. 1979. Induced spawning of pond-reared milkfish, Chanos chanos Forsskal. China Fish. Monthly 330: 7-13 (in Chinese with English abstract).

Kumagai, S., N.M. Castillo, and V.C. Banada. 1978. Spawning periodicity of milkfish Chanos chanos. Quarterly Res. Rep. SEAFDEC Aquacult. Dept. 2(2): 10-12.

Kumagai, S. 1984. The ecological aspects of milkfish fry occurrence, particularly in the Philippines. Proc. 2nd Int. Milkfish Aquaculture Conf., Iloilo, Philippines, Oct. 4-8, 1983. pp. 53-68.

Kuo, C.M. 1982. Progress on artificial propagation of milkfish. ICLARM Newsletter 5 (1): 8-10.

Kuo, C.M. and C.E. Nash. 1979. Annual reproductive cycle of milkfish, Chanos chanos Forskal, in Hawaii waters. Aquaculture 16: 247-251.

Kuo, C.M., C.E. Nash, and W.O. Watanabe. 1979. Induced breeding experiments with milkfish, Chanos chanos (Forskal), in Hawaii. Aquaculture 16: 247-252.

Kuronuma, K. and M. Yamashita. 1962. Milkfish fry in eastern coast of Vietnam. J. Oceanogr. Soc. Japan. 20th Anniversary Vol. 679-686.

Lacanilao, F., C.L. Marte and T.J. Lam. 1985. Problems associated with hormonal induction of gonad development in milkfish (Chanos chanos). In: B. Lofts and W.N. Holmes (Eds.), Current Trends in Comparative Endocrinology. Hong Kong University Press, Hong Kong. pp. 1247-1253.

Lacanilao, F.L. and C.L. Marte. 1980. Sexual maturation of milkfish in floating cages. Asian Aquaculture 3: 4-6.

Lam, T.J. (Ed.) 1986. Control of Reproduction in Fish: A Roundtable Discussion. International Development Research Centre Publication Series MR-123e.

Lee, C.S. 1985. Environmental factors in the reproduction of milkfish. In: C.S. Lee and I.C. Liao (Eds.) Reproduction and Culture of Milkfish. Oceanic Institute, Hawaii and Tungkang Marine Lab., Taiwan. pp. 99-114.

Lee, C. S., C.S. Tamaru, J.E. Banno, C.D. Kelley, A. Bocek and J.A. Wyban. 1986a. Induced maturation and spawning of milkfish, Chanos chanos Forsskal, by hormone implantation. Aquaculture 52: 199-205.

Lee, C.S., C.S. Tamaru, J.E. Banno, and C.D. Kelley. 1986b. Influence of chronic administration of LHRH-analogue and/or 17α-methyltestosterone on maturation in milkfish, Chanos chanos. Aquaculture (in press).

Lee, C.S., C.S. Tamaru, C.D. Kelley and J.E. Banno. 1986c. Induced spawning of milkfish, Chanos chanos, by a single application of LHRH-analogue. Aquaculture (in press).

Lee, C.S., G. M. Weber, and C.S. Tamaru. 1986d. Effects of orally administered 17α-methyltestosterone on spermatogenesis in immature milkfish, Chanos chanos (Forskal). J. Fish Biology (in press).

Lee, C.S. and G.M. Weber. 1983. Preliminary studies on the maturation of milkfish Chanos chanos in an environmentally controlled system. Presented at the 2nd Int. Milkfish Aquaculture Conf., Iloilo, Philippines, Oct. 4-8, 1983.

Liao, I.C. 1971. Notes on some adult milkfish from the coast of southern Taiwan. Aquaculture 1(3): 1-10. (In Chinese with English abstract).

Liao, I.C. and T.I. Chen. 1979. Report on the induced maturation and ovulation of milkfish (Chanos chanos) reared in tanks. Proc. World Maricul. Soc. 10: 317-331.

Liao, I.C. and T.I. Chen. 1983. Report on the gonadal development of milkfish, Chanos chanos, reared in tanks. Aquiculture 2.

Liao, I.C. and T.I. Chen. 1984. Gonadal development and induced breeding of captive milkfish in Taiwan. In: J.V. Juario, R.P. Ferrari and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Metro Manila, Philippines. pp. 41-51.

Liao, I.C., J.V. Juario, S. Kumagai, H. Nakajima, M. Natividad and P. Buri. 1979. On the induced spawning and larval rearing of milkfish, Chanos chanos (Forsskal). Aquaculture 18: 75-93.

Lin, L.T. 1984. Studies on the induced breeding of milkfish (Chanos chanos Forskal) reared in ponds. China Fisheries No. 378: 3-29.

Lin, L.T. 1985. My experience in artificial propagation of milkfish -- Studies on natural spawning of pond-reared broodstock. In: C.S. Lee and I.C. Liao (Eds.) Reproduction and Culture of Milkfish. Oceanic Institute, Hawaii and Tungkang Marine Laboratory, Taiwan. pp. 185-203.

Marte, C.L. and L.W. Crim. 1983. Gonadotrophin profiles in serum of milkfish treated with salmon pituitary homogenate. Kalikasan, Philip. J. Biol. 12(1-2): 100-106.

Marte, C.L., F.J. Lacanilao and J.V. Juario. 1983. Completion of the life cycle of milkfish Chanos chanos (Forskal) in captivity. Presented at 2nd Int. Milkfish Aquaculture Conf., Iloilo, Philippines, Oct. 4-8, 1983.

Marte, C.L., G.F. Quinitio, L. Ma. B. Garcia, and F.J. Lacanilao. 1984. A guide to the establishment and maintenance of milkfish broodstock. SEAFDEC-AQD, IDRC Tech. Rep. 11 pp.

Marte, C.L. and T.J. Lam. 1985. Seasonal and diurnal serum levels of estradiol-17β and testosterone in milkfish (Chanos chanos). Presented at 2nd Int. Conf. Warmwater Aquaculture -- Finfish. Laie, Hawaii. 1984.

Olsson, R. 1974. Fine structure of the pituitary eta cells of larval Chanos chanos Teleostei. Gen. Comp. Endocrinol. 22(3): 364.

Schuster, W.H. 1960. Synopsis of biological data on milkfish Chanos chanos (Forskal), 1975. FAO Fish. Biol. Synop. (4), 64 pp.

Senta, T., S. Kumagai and N. Castillo. 1980. Occurrence of milkfish, Chanos chanos (Forskal) eggs around Panay Island, Philippines. Bull. Fac. Fish. Nagasaki Univ. 48: 1-11.

Sherwood, N.M., B. Harvey, M.J. Brownstein and L.E. Eiden. 1984. Gonadotrophin-releasing hormone (Gn-RH) in striped mullet (Mugil cephalus), milkfish (Chanos chanos), and rainbow trout (Salmo gairdneri): Comparison with salmon Gn-RH. Comp. Endocrinol. 55: 174-181.

Smith, A.C. 1978. Immunological reactions of the sea cucumber, *Holuthuria cinerascens*, to serum from the milkfish, *Chanos chanos*. J. Invertebr. Pathol. 29(3): 326-331.

Tampi, P.R.S. 1951. Pituitary of *Chanos chanos*. Nature (London) 167: 686-687.

Tampi, P.R.S. 1953. On the structure of the pituitary and thyroid of *Chanos chanos* (Forskal). Proc. Nat. Inst. Sci. India. 19(2): 247-256.

Tan, J.D. 1982. A histological study of the hypophysial-gonadal system during sexual maturation and spawning in the milkfish *Chanos chanos* Forskal. M.S. Thesis to Univ. of Philippines. 41 pp.

Tan, J.D. 1985. A histological study of the hypophysial-gonadal system during sexual maturation and spawning in the milkfish *Chanos chanos* Forskal. J. Fish. Biol. 26: 657-668.

Wainwright, T. 1982. Milkfish fry seasonality on Tarawa, Kiribati, its relationship to fry seasons elsewhere, and to sea surface temperatures (SST). Aquaculture 26: 265-271.

Yamazaki, F. 1965. Endocrinological studies on the reproduction of the female goldfish *Carassius auratus* (L.) with special reference to the function of the pituitary gland. Mem. Fac. Fish. Hokkaido Univ. 13: 1-64.

4. ARTIFICIAL PROPAGATION

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4-1. INTRODUCTION

Milkfish farmers have traditionally depended on natural recruitment to provide seed stock for their ponds. However, the quantity of these fry undergoes yearly fluctuations, resulting in the destabilization of the milkfish farming industry. Efforts to artificially propagate milkfish have been undertaken in order to control the supply of fry.

Artificial propagation involves inducing captive milkfish to mature and spawn in an artificial environment, such as a tank or pond. Two major problems have plagued attempts to produce fry under these conditions. First, milkfish require a minimum of around five years to reach sexual maturity (Lam, 1984; Liao and Chen, 1984). Second, sexually mature broodstock usually undergo gonadal atresia in captivity, instead of completing seasonal rematuration. This may result from stress during capture or maintenance in an artificial environment.

Progress toward solving these problems forms the basis of this chapter. Capturing sexually mature milkfish or attempting to hormonally induce sexual maturity in immature fish are two approaches to the first problem. One approach to the second problem is to improve capture and management techniques to obtain natural maturation and spawning in captivity. Another is to induce maturation and spawning through either environmental or hormonal control. This chapter begins by describing current techniques for obtaining and handling broodstock, followed by a review of what is presently known about proper broodstock management. The remainder of the chapter focuses on both natural and induced maturation and spawning.

4-2. ACQUISITION OF BROODSTOCK

4-2.1. WHEN AND HOW TO CAPTURE

Milkfish are captured year round to obtain potential broodstock. Fish captured during the off-season do not require hormone treatment to prevent gonadal atresia and can be acclimated to the holding facility prior to the onset of the reproductive season. These advantages have been useful for induced maturation attempts (Lee et al., 1986b). There are, however, major disadvantages to obtaining the fish when their gonads are in a regressed state. First, the sex of the individuals cannot be determined; therefore, the males and females cannot be immediately segregated or stocked at specific ratios. Second, sexually mature fish cannot be distinguished from sexually immature fish. Size and weight are poor indicators of sexual maturity in wild-caught milkfish since mature fish can weigh as little as 1 kg (Crear, 1980). Most of the broodstock which have spawned naturally or have been induced to spawn, however, have weighed 3 kg or more (Lam, 1984; Lin, 1985; Lee et al., 1986c). This weight may therefore provide a rough estimate of sexual maturity. In Hawaii, an additional disadvantage is that regressed mature or immature milkfish usually do not enter a water gate and are rarely captured with this method (Uyemura, pers. comm.).

For induced spawning attempts, milkfish are captured during the reproductive season (Vanstone et al., 1977). Crear (1980) found evidence of a lunar spawning periodicity at Christmas Island, which suggests that a greater number of wild mature females with large eggs may be obtained one week before the full moon. In the Philippines, however, spawning appears to be most intense around the quarter-moon periods (Kumagai, 1984). In general, a major advantage to capturing milkfish "in season" is that a cannula can sample mature gametes to verify both sexual maturity and sex. The disadvantage is that gonadal atresia may result from capture stress. This problem, however, has been overcome through the use of hormones or tranquilizers (see Section 4-2.2).

Milkfish are found in a variety of habitats, including shallow and deep open water, as well as hyposaline, normal saline, and hypersaline enclosed water (i.e., saltwater ponds or lakes). The characteristics of a particular habitat--water depth, bottom profile, and degree of enclosure--will determine the appropriate method to use for capturing potential broodstock. Kuo (1982) states that a 200 m X 6.6 cm mesh monofilament gill net is effective in any habitat. The net must be constantly monitored, however, since the fish are frequently injured from entanglement. Seine nets inflict less injury to the fish (Kuo et al., 1979), but their use is restricted to shallow bodies of water, preferably those partially or completely enclosed.

A variety of fish traps are found suitable for capturing milkfish. "Otoshiami" (a kind of net) have proven effective in deeper open water and have been used during the milkfish migration to the coast of Panay Island in the Philippines (Chaudhuri et al., 1978). Once inside, the fish are removed using a fine mesh scoop net and placed into a floating cage which is then towed to shore. On Mindoro Island, a "barrage" type fish trap was used during the milkfish migration from Lake Naujan to the sea. "Corral" type fish traps have been used successfully in open or large enclosed bodies of water that are either shallow or shallow on one side (Juario and Duray, 1983).

A water gate provides an effective method of capturing milkfish from ponds during the reproductive season (Fig. 1). During tidal changes, mature milkfish attempt to migrate through the water gate to the sea. Once inside the gate, the fish are easily captured by securing the second screen in place (Uyemura, pers. comm.).

4-2.2. HANDLING, TRANSPORTING AND INITIAL TREATMENT

Once captured, milkfish are usually transferred either directly to the holding facility, if close enough, or to a transport tank. Proper handling of the fish during these transfers minimizes injury. Scoop nets have been used for

Fig. 1. Water Gate, Big Island, Hawaii.

distances of several feet without any apparent ill effects. Fish have also been placed in plastic bags filled with water and carried short distances either by hand (Fig. 2) or on a stretcher (Chaudhuri et al., 1978; Lee et al., unpub.).

Milkfish have been transported long distances using transport tanks. Lee et al. (1986b) captured 177 milkfish on the island of Hawaii and transported them to the island of Oahu using a transport tank 0.6 m deep with a surface area of 5.2 m^2 (Fig. 3). All the fish were 6 to 8 years of age with an average body weight of 2.16 kg. Fish were stocked at a density of 1/0.26 m^2 (20 per tank). The water was clean pond water with a salinity between 7 and 10 ppt. Bags of ice were placed inside with the fish to keep the water temperature low during transit. The tank was fitted with two oxygen bottles, one set at a moderate flow rate, while the other was set at a low flow rate and served as a backup. The transports lasted from 18 to 28 hours. At completion of the longest, the NH_4,

Fig. 2. Milkfish transfer bag, Hawaii.

Fig. 3. Transport tank, Hawaii.

NO_3/NO_2, and PO_4 levels were 0.64, 0.17, and 0.06 mg/liter, respectively. One hundred fifty (85%) of the fish survived the transport.

In the Philippines, SEAFDEC uses a 2 m diameter, 1 m deep transport tank when transporting fish between facilities (Banno, pers. comm.). The surface area is 3.14 m^2 and usually only 5 fish are transported at a time, either by boat or by truck.

Broodstock are generally treated for possible injury or stress after being transported or handled. The adipose tissue covering the eyes is commonly injured and becomes opaque shortly after handling (Fig. 4). This problem usually heals itself, although more serious cases can result in mortality due to severe infection or as an indirect consequence of blindness. Kuo et al. (1979) found that maintaining newly-caught fish in a large holding tank with adequate water exchange and treating them with furacin or

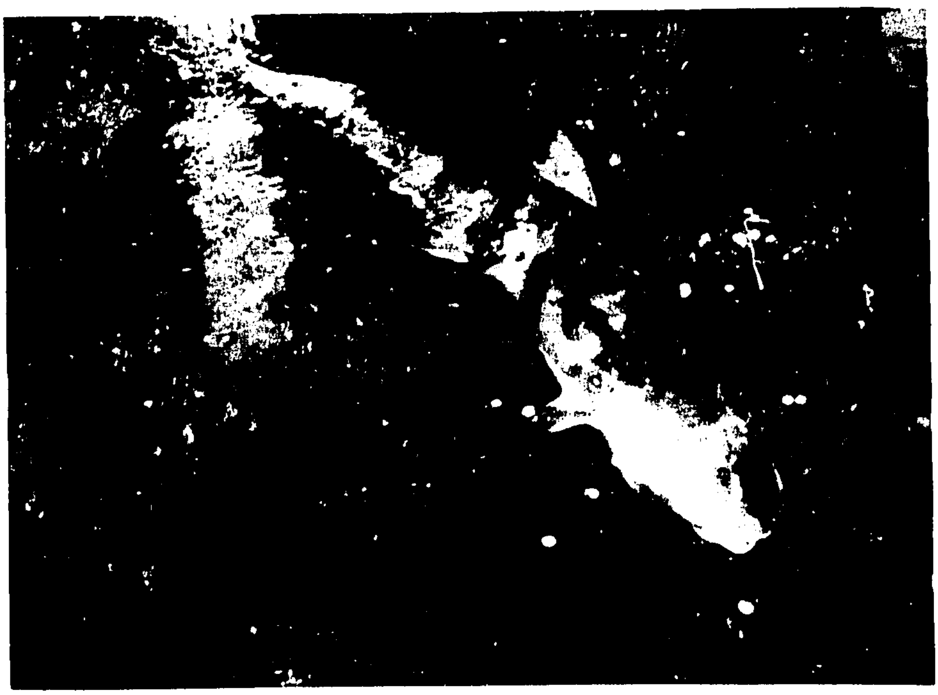

Fig. 4. Opaque adipose tissue on milkfish in Hawaii.

furan 2 greatly decreased the incidence of infection in general. Juario et al. (1984) suggested the use of tetramycin ointment. Another common practice is to lower the water salinity in the holding tank.

In response to handling stress, mature females with large vitellogenic oocytes often undergo gonadal atresia (Lam, 1984). Kuo et al. (1979) prevented atresia by injecting the fish with a minimum dose of 1.7 mg partially purified salmon gonadotropin (SG-G 100)/kg body weight or 5.56 mg of carp pituitary homogenate (CPH) combined with 555.5 International Units (IU) human chorionic gonadotropin (HCG)/kg body weight within 26 hours of first handling. They recommended, however, that these injections be given immediately after capture. Juario et al. (1984) used injections of gonadotropin, preferably administered within the first 6 hours. Kuo (1982) successfully used the tranquilizer Valium (diazepam) to reduce the stress of capture and therefore the incidence of atresia.

An increase in milt viscosity is a common problem in mature males subjected to handling stress. Juario et al. (1980) successfully used Durandron Forte 250 to maintain running condition following capture.

Kuo et al. (1979) recommend that mature females captured from either brackish or hypersaline ponds be acclimated slowly to a salinity of 35 ppt before attempting to induce spawning. They found that females who have been acclimated slowly respond better to hormone treatments. The acclimation was made by maintaining the fish at the same level of salinity for several hours, then gradually increasing or decreasing the salinity to 35 ppt at the rate of 1-4 ppt per hour.

4-3. MANAGEMENT OF BROODSTOCK

Broodstock management, as practiced in the Philippines, has been reviewed by Marte et al. (1984b). Table 1 presents dimensions of currently operating holding facilities, stocking densities, and sex ratios reported or estimated from reports of maturation or spawning of milkfish in the Philippines, Taiwan and Hawaii. Table 2 presents the reported water temperatures and salinities in which milkfish are known to have either matured or spawned.

4-3.1. HOLDING FACILITIES

The ideal management strategy is to maintain broodstock in an enclosure where they naturally mature and spawn fertilized eggs which are retained and easily collected for incubation, hatching and larval rearing. This strategy has been realized in only one holding facility: Lin's dirt ponds in Taiwan (Lin, 1985). Natural maturation and spawning have taken place in three of his ponds, which are rectangular and have dimensions of 25 X 30 X 1.3 m deep or 30 X 50 X 1.5 m deep (Fig. 5a). Each pond is provided with a paddlewheel aerator and water exchange system. The aerator also helps circulate water which facilitates the collection of eggs by a large, stationary plankton net.

Natural maturation and spawning have only occurred in one other facility: SEAFDEC's floating cages in the Philippines (Lacanilao and Marte, 1980; Marte et al., 1984a; 1984b). Each cage is circular and has a diameter of 10 m and a depth of 3 m. The mesh size is 5.7 cm on the sides and 3.0 mm on the bottom to retain feed. The facility is located in a natural cove, protected by small islands. The water depth is approximately 7 m. One advantage to this type of facility is that no artificial aeration or water exchange system is required. A drawback is that the majority of spawned eggs are not retained within the cages, and are therefore not collected. This problem, however, is being addressed.

Natural spawning has not taken place in any other facilities. Furthermore, the percentage of broodstock which naturally matures is quite low (Liao and Chen, 1979). An

Table 1. Dimensions, stocking densities and sex ratios of various holding facilities.

	Holding Facility	Surface Area (m^2)	Depth (m)	# of Fish/ Facility	Stocking Density (m^2/Fish)	Sex Ratio (male/female)	Reference
MATURATION							
NATURAL	Dirt Ponds	750	1.3	30	25.0	1.0	Lin, 1985
		1500	1.5	50	20.0	1.0	Lin, 1985
		500	1.0	12	42.0	?	Kuo, 1982
	Floating Cages	79	3.0	108	0.7	?	Marte et al., 1984a,b
	Fiberglass Tanks	30	1.2	10	3.0	0.43-3.5	Lee et al., 1986b
	Cement Tanks	57-113	1.2	20-30	2.9-5.7	?	Liao & Chen. 1979
		44	1.3	14	3.1	?	Lee, unpub.
INDUCED							
Photoperiod	Cement Tank	25	1.3	6-7	2.3-3.6	0.83	Lee & Weber, 1983
Photo/Hormone	Cement Tank	25	1.3	5	2.3	0.83	Lee et al.(in press)
Hormone	Fiberglass Tanks	30	1.2	10	3.0	3.50	Lee et al., 1986b
	Cement Tank	44	1.3	16	2.7	0.43	Lee et al., 1986b
SPAWNING							
NATURAL	Dirt Ponds	750	1.3	30	25.0	1.0	Lin, 1985
		1500	1.5	50	20.0	1.0	Lin, 1985
	Floating Cages	79	3.0	108	0.7	?	Marte et al., 1984a,b
INDUCED							
Hormone	Fiberglass Tanks	6.8	0.8	3-4	2.2-2.9	2-3	Lee et al., 1986c

Table 2. Water temperatures and salinity.

	Water Temperature (^oC)	Water Salinity (ppt)
<u>Maturation</u>		
Natural	21-32	8-130
Induced		
Photoperiod	25-27	30-36
Hormone	22-29	32-38
<u>Spawning</u>		
Natural	25-30	28-130
Induced		
Hormone	25-30	32-39

alternative management technique has therefore been adopted by other facilities. Broodstock are maintained in maturation facilities where they naturally mature, or they are induced to mature with hormones or controlled photoperiods. Once the female broodstock's oocytes have reached the appropriate size, the females are transferred to separate spawning tanks, each with or without one or more mature males. Final maturation and ovulation are then induced with the use of various hormones (see Sections 4-7 and 4-8). Subsequently, the females either spawn spontaneously or are stripped of their eggs for <u>in vitro</u> fertilization (i.e., strip spawning).

The types of maturation facilities in which broodstock have naturally matured include circular cement tanks in Taiwan (Liao and Chen, 1979; Tseng and Hsiao, 1979) and square cement tanks, circular fiberglass tanks and dirt ponds in Hawaii (Kuo, 1982; Lee et al., 1986b; Lee, unpub.).

The circular tanks in Taiwan are located at Tungkang Marine Laboratory and are between 8.5 and 12 m in diameter


Fig. 5a. Lin's ponds, Taiwan

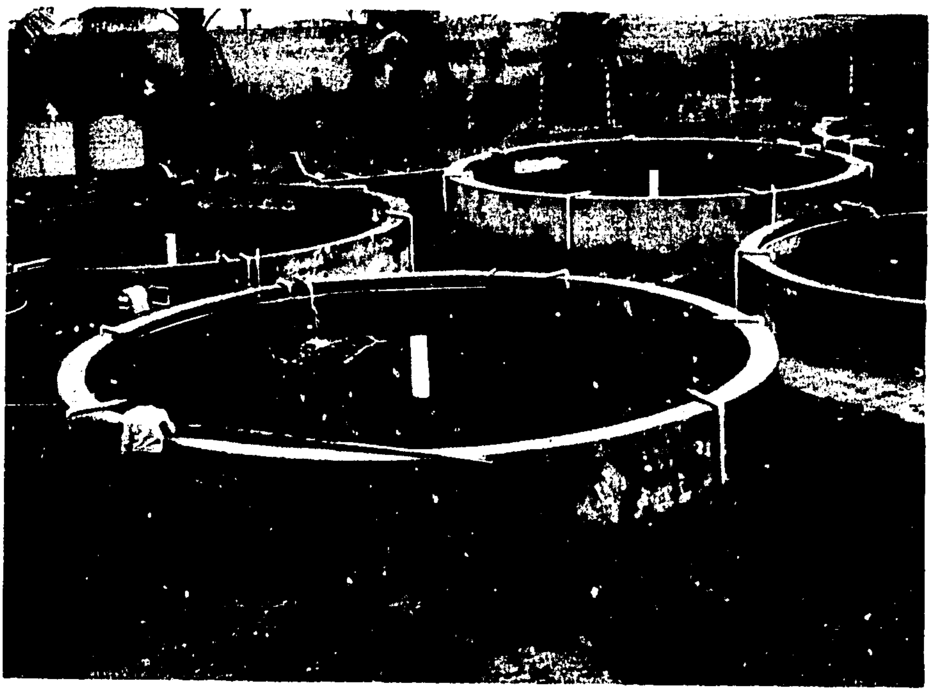
Fig. 5b. Circular cement tanks, Taiwan.

with a depth of 1.2 m (Fig. 5b). All tanks are provided with water exchange and aeration.

The maturation tanks in Hawaii are located at the Oceanic Institute. The square cement tank is outdoors but covered by an opaque fiberglass roof (Fig. 5c). Its dimensions are 6.7 X 6.5 X 1.3 m deep (Fig. 5c) and a water exchange and aeration system is provided. The circular fiberglass tanks have a diameter of 6.14 m and a depth of 1.2 m (Fig. 5d). They are also provided with water exchange and aeration. The dirt ponds have surface areas of 500 m^2 and depths of 1 to 2 m (Fig. 5e). All have a water exchange system but are not aerated.

Maturation has been hormonally induced in the square outdoor cement tank and circular fiberglass tanks (Lee et al., 1986b). Maturation has been induced by controlled photoperiod in a square indoor cement tank (Fig. 5f) measuring 5.2 X 4.8 X 1.3 m deep (Lee and Weber, 1983, Lee et al., in press). This tank has both water exchange and aeration systems along with eight 2.4 m long, full spectrum fluorescent light bulbs suspended 1.2 m above the surface and connected to a 24-hour timer. A combination of hormone treatments administered simultaneously with a controlled photoperiod has also been successfully used on fish maintained in this tank (Lee et al., in press).

The types of spawning tanks where spontaneous spawning has taken place following hormone treatment include small fiberglass tanks at the Oceanic Institute (Lee et al., 1986c) and a holding tank in Taiwan (Lin, 1984). The former are circular and have a diameter of 3.4 m, a depth of 0.8 m, and are provided with water exchange and aeration systems (Fig. 6). A central overflow pipe is fitted with fine mesh nylon to retain spawned eggs. Following a spawn, the eggs are removed with hand nets and transferred to incubation tanks. A description of the holding tank in Taiwan was not reported.

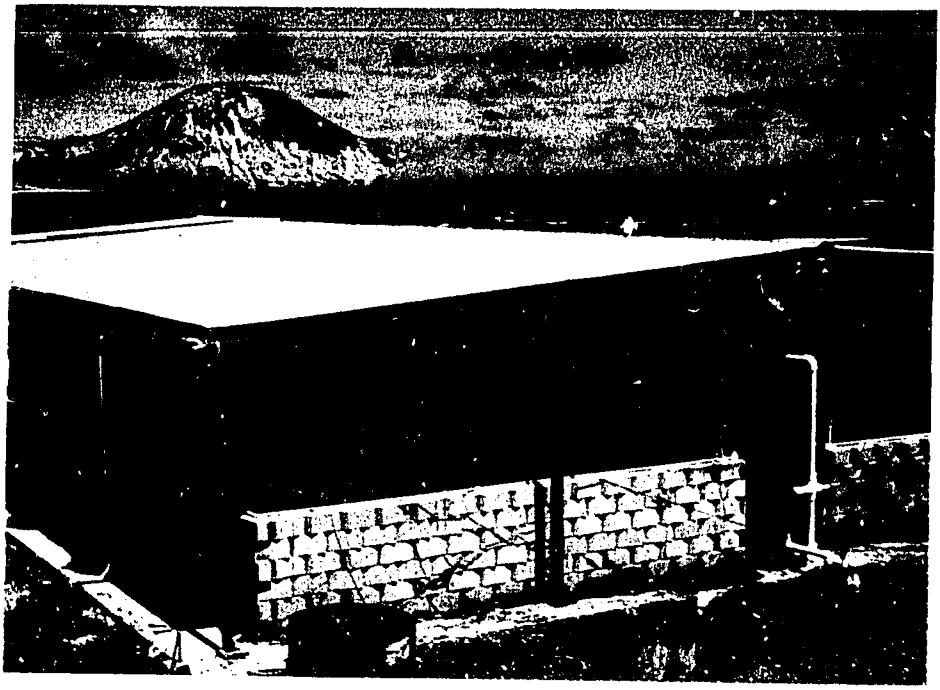

Fig. 5c. Covered cement tanks, Hawaii.

Fig. 5d. Circular fiberglass tanks, Hawaii.

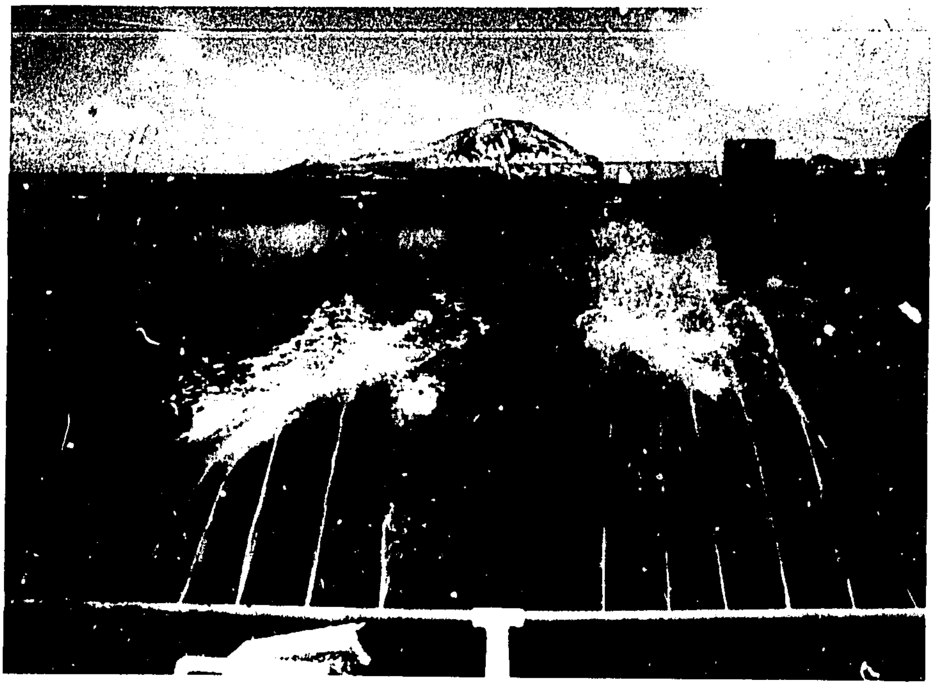

Fig. 5e. Dirt ponds, Hawaii.

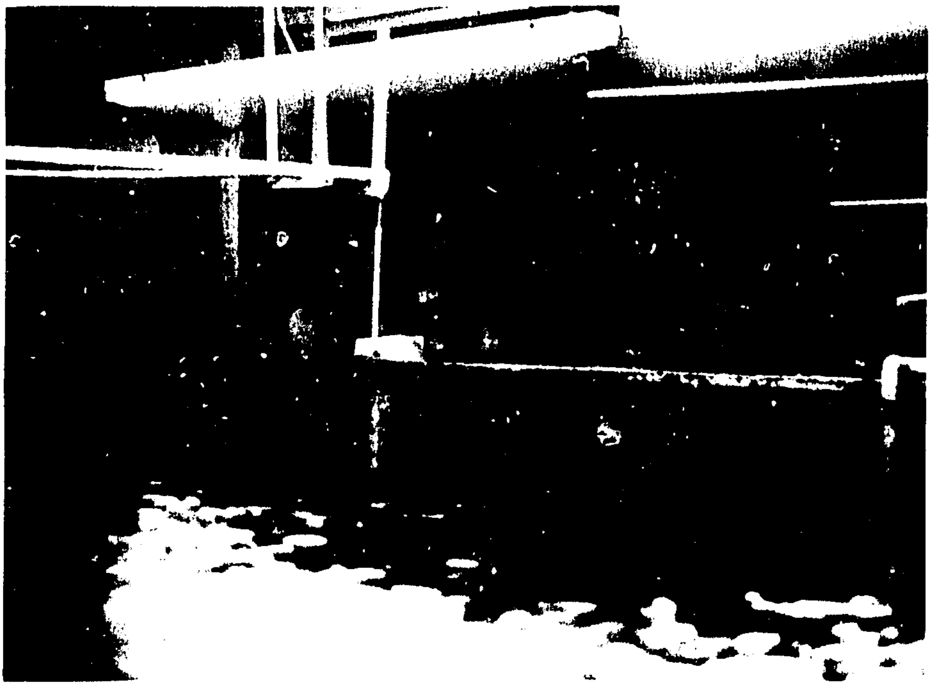

Fig. 5f. Photoperiod tank, Hawaii

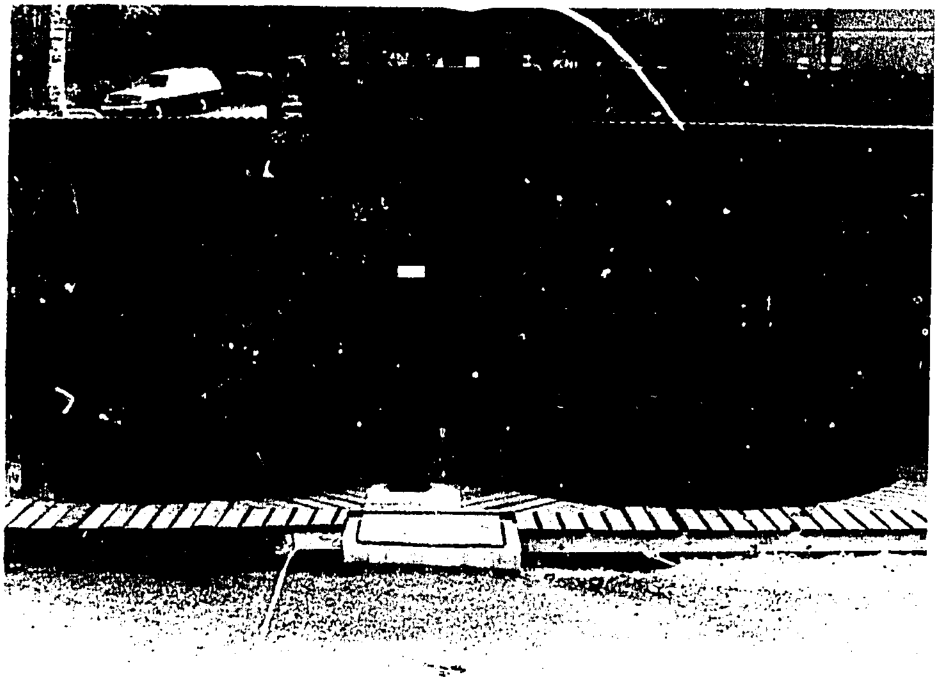

Fig. 6. Spawning tanks, Hawaii.

Both a 4 m diameter and a 6 m diameter canvas tank have been used in the Philippines to hold females prior to strip spawning (Banno, pers. comm.).

4-3.2. STOCKING DENSITY AND SEX RATIO

Most of the cases of natural maturation and spawning have taken place at relatively low stocking densities, 20 to 42 m^2/fish, and at a 1:1 sex ratio (Lin, 1985). Natural spawning has reportedly taken place, however, at a density of 0.7 m^2/fish in sea cages (Lacanilao and Marte, 1980).

In maturation tanks and ponds, the stocking densities vary between 2.3-5.7 m^2/fish, while known sex ratios range between 0.43-3.5 males/female (Liao and Chen, 1979; Kuo, 1982; Lam, 1984; Lee, unpub.; Lee and Weber, 1983; Lee, 1985; Lee et al., 1986b).

Spontaneous spawning has been induced at stocking densities of 2.2-3.0 m^2/fish and with sex ratios between 0.4-3.0 males to females with 2:1 being the most common (Kuo, 1984;

Lee et al., 1986c).

4-3.3. TEMPERATURE AND SALINITY

Natural maturation and spawning in Taiwan and the Philippines have taken place in temperatures varying between 25-30°C and in salinities ranging between 28-39 ppt (Lacanilao and Marte, 1980; Lin, 1985). At Christmas Island, however, milkfish spawned in landlocked lagoons where the salinity can be as high as 130 ppt (Crear, 1980).

The temperature and salinity in maturation tanks and ponds have ranged between 21-32°C and 8-38 ppt, respectively (Lam, 1984; Lee et al., 1986b; Liao and Chen, 1984; Marte et al., 1984b). Induced spawning has taken place in temperatures ranging between 25-30°C and in salinities ranging between 32-39 ppt (Lee et al., 1986c).

Lam (1984) summarized current knowledge on the effects of temperature and salinity on gonadal maturation and spawning. Lee (1985) also reviewed the relationship between various environmental factors and the reproduction of milkfish. The environmental cues for maturation and spawning have yet to be defined, however. Maturation can occur in a wide range of salinities and may be stimulated by seasonal increases in water temperature and daylight hours (Marte et al., 1984b). Spawning has been reported only at salinities at or above normal seawater and may be inhibited by rapid changes in salinity (Kuo et al., 1979). Wainwright (1982) suggested that spawning can take place only above 24°C.

4-3.4. DIET

Lin (1985) fed his broodstock a variety of feeds including rice bran, wheat meal, soybean meal, formulated eel feed and trash fish. Milkfish that matured and spawned naturally in SEAFDEC's floating cages were fed crustacean feed pellets containing 42% protein (Lacanilao and Marte, 1980). Natural and induced maturation, followed by either spawning or stripping, has also occurred in milkfish fed

Purina Trout Chow and compound feeds comprised of 32-46% protein (Lee et al., 1986b; Liao and Chen, 1984). Lam (1984) suggested that a high protein diet may be important for milkfish maturation and spawning. Crear (1980) found that brine shrimp were a major component of the diet of mature milkfish at Christmas Island.

4-4. NATURAL MATURATION

4-4.1. SEXUAL MATURITY

Liao and Chen (1984) summarized current knowledge concerning sexual maturity in milkfish. Mature sperm have only been found in males who are 4 or more years old. Vitellogenesis has been found to occur only in females 5 years or older. The factors controlling sexual maturity have not been identified and it is presently unknown whether sexual maturity can be accelerated under certain conditions. In Hawaii, sexual maturity has been retarded in milkfish (Lee et al., 1986d). Although these fish were 6 years old, they weighed only 1.4 kg in comparison to the estimated mature size of 3 kg or more (see Section 4-2.1).

It is assumed that under normal conditions, once milkfish reach sexual maturity, maturation will depend on the same factors important for seasonal rematuration.

4-4.2. SEASONAL REMATURATION

After maturation is initiated in females, vitellogenesis can proceed until the oocytes reach a diameter of 950 μm (Lee et al., in press). Completion of maturation is defined here, however, as the attainment of the point where final maturation can be induced which coincides with an oocyte diameter of approximately 660 μm (Juario et al., 1984; Lam, 1984). Only eight of every twenty broodstock in Taiwan begins rematuration naturally, of which one or two actually complete the process (Liao and Chen, 1984). As Lam (1984) pointed out, it is difficult to isolate the factors important to maturation because milkfish mature in a variety of facilities under different conditions. Marte et al. (1984b) suggested that a seasonal increase in water temperature and photoperiod stimulated maturation. Lee et al. (in press) suggested that photoperiod alone was the important factor. A high protein diet which includes brine shrimp has also been suggested as a factor (Crear, 1980; Lam, 1984). Lin (1984, 1985) reported numerous incidents of natural maturation and spawning in ponds where the stocking density was relatively low compared to other facilities. All of these factors need more experimental study, however, before their importance to natural maturation can be confirmed (Lam, 1984).

4-5. INDUCED MATURATION

The basic assumption behind attempts to induce either maturation or spawning in milkfish is that their capture or maintenance in an artificial environment has adversely affected the synthesis or release of hormones at some point along the hypothalamic-pituitary-gonad (H-P-G) axis. Two approaches have been used to counter this apparent block in the H-P-G axis: alleviating the block through environmental manipulation (i.e. photoperiod control) or bypassing the block using various analogues of either hypothalamic-releasing factors (LHRH-a), pituitary gonadotropins (salmon pituitary homogenate [SPH], SG-G 100, CPH, HCG) or gonadal steroids (estradiol-17β [E_2], Durandron Forte, [a long-acting testosterone preparation], testosterone, or 17α-methyltestosterone [17-MT]). These hormones have been administered using a variety of vehicles: oral food pellets, intramuscular (IM) injection, IM and intraperitoneal (IP) pellet implants and IP infusion.

This section describes attempts that have been made using either an environmental or hormonal approach to induce either sexual maturity or seasonal rematuration.

4-5.1. SEXUAL MATURITY

Lacanilao et al. (1984) attempted to induce sexual maturity in immature fish that were between 3 and 5 1/2 years old and weighed between 1.6 and 3.7 kg. The hormones used in these experiments included SPH, SG-G100, HCG, L-thyroxine sodium (T_4), E_2, and Durandron Forte. These hormones were administered in varying dosages, either alone or in combination, by IM injection, IM or IP cholesterol pellet implantation or IP infusion.

All treatments were unsuccessful in inducing sexual maturity in either males or females. Factors thought to explain this lack of response include stress, the unsustained release of hormone from pellets, the size of holding facilities, unstable environmental conditions, inappropriate hormone dosages, inadequate methods or frequency of hormone administration, and the possible production of anti-hormones after prolonged treatment. Lam (1984) suggested that milkfish less than 4 or 5-years-old may not have developed the receptors to respond to hormone treatments.

Lee et al. (1986d) attempted to induce sexual maturity in male milkfish through oral administration of 17-MT. Although these fish were of mature age, sexual maturity had been retarded (see Section 4-4.1). Fifty-four 5-year-old fish and 48 6-year-old fish with average body weights of 1.4 and 1.7 kg, respectively, were fed Purina Catfish Chow which was sprayed with 17-MT dissolved in ethanol or mixed with 17-MT dissolved in codliver oil. Dosages varied between 6.25 mg/kg body weight to 25.0 mg/kg body weight. The addition to the diet of 17-MT, dissolved in either codliver oil or ethanol, induced significantly higher GSIs than in the control fish. The testes of treatment fish contained sperm in all stages of development. Even though no running milt was exuded from any male, these results do suggest that sexual maturity can be induced in male milkfish. The optimal dosage suggested was 12.5 mg/kg body weight per day.

4-5.2. SEASONAL REMATURATION - PHOTOPERIOD CONTROL

Lee and Weber (1983) attempted to induce maturation in seven milkfish by switching from a photoperiod regime of 6L:18D to 12L:12D to 18L:6D for intervals of three months each. The broodstock were 7 years old and had been acclimated to the tank for four years. Four mature females were found in May and June of 1982, with average sizes of oocytes ranging from 840 to 940 µm. The two females with the largest average oocyte diameters (920 and 940 µm) showed signs of gonadal atresia. All four females were subsequently used in hormone-induced final maturation experiments.

Two males were also found to have matured in June, one of which was used to fertilize the stripped eggs of one of the females mentioned above.

Lee et al. (in press) attempted to induce maturation using photoperiod control. Six milkfish were subjected to a photoperiod regime similar to that mentioned above. Two females and three males subsequently matured. The females matured at the beginning of May with average oocyte diameters of 788 and 835 µm. Mature sperm were found in the three males in July and August. In comparison to milkfish maintained in an outdoor tank with exposure to the ambient photoperiod, a larger percentage of the fish exposed to controlled photoperiods matured.

4-5.3. SEASONAL REMATURATION - PHOTOPERIOD/HORMONAL CONTROL

Lee et al. (in press) attempted to induce maturation in five milkfish using controlled photoperiods in conjunction with 200 µg LHRH-a cholesterol pellet implants. The photoperiod regimes were the same as mentioned above. Four of the five fish matured. Two females were found to have oocyte diameters of 950 and 853 µm in April, one month earlier than females exposed to controlled photoperiods without implants.

4-5.4. SEASONAL REMATURATION - HORMONAL CONTROL

In addition to experiments with sexually immature fish, Lacanilao et al. (1984) attempted the same hormone treatments with wild regressed milkfish weighing between 4.9 and 12.0 kg. As in the case of the sexually immature fish, these experiments were unsuccessful in triggering the onset of maturation.

Lam (1982) attempted to induce the completion of maturation followed by spawning in a recently-caught, wild female weighing 8 kg and having oocytes with an average diameter of 570 µm. The hormones used were E_2 and T_4, in combination with SP^{H} and HCG. The results were promising: the oocytes increased to 660 µm in diameter. The female died after the third injection, however.

Lee et al. (1986a and b) recently induced maturation and spawning one to two months prior to the reproductive season by IM implantation of an LHRH-a cholesterol pellet, either alone or in combination with a 17-MT silastic capsule. The initial state of the ovaries and testes was not assessed for all fish, however, because of concern over handling stress. It is unclear, therefore, whether the hormone treatment induced the onset or enhanced the completion of maturation.

In their experiments, nineteen 6 to 8-year-old milkfish, ranging in size from an estimated 2.5 to 6.1 kg, were implanted with a 200 µg LHRH-a cholesterol pellet once a month between March and July of 1985. A 250 µg 17-MT silastic capsule was implanted in March and another in June, 1985. Eight females were induced to complete maturation one to five times during the six-month period and oocytes grew from 250 to 755 µm or more in diameter in one to two months. These females were subsequently induced to spawn 15 times for a rate of 1.75 spawns per female. One female spawned five times. Oocytes that did not undergo final maturation became atretic, and we believe that at least five additional spawns could have been induced if the females were monitored more frequently than once a month.

All 10 males achieved a running ripe condition during the six-month period. Eight of the 10 were found to be running ripe in April, one month after the first implantation and were maintained in this condition through August. Of 19 fish, only one remained negative throughout the six-month period.

4-6. NATURAL SPAWNING

As mentioned earlier, although natural maturation of broodstock has taken place in a wide variety of facilities and conditions, natural spawning has taken place in only two facilities: Lin's ponds and SEAFDEC's floating cages. The holding facilities, stocking rates, environmental conditions and diets involved have already been described. This section provides additional specific details concerning both these cases.

<u>4-6.1.</u> <u>LIN'S</u> <u>PONDS</u>

The first natural spawns in Lin's ponds took place in October of 1983 (Lin, 1984). No data are available on this spawn aside from the ages of the fish, which were 9 to 10-years-old. In 1984, Lin recorded a total of 62 spawns between April and September (Lin, 1985). The fish were 10 to 11-years-old and ranged in size from 3 to 7.5 kg. Even though all the fish were from the same stock, 56 of the spawns took place in ponds where all the broodstock were over 4 kg in weight, suggesting that size as well as age may be a factor. The number of eggs collected per spawn varied from a few to 7,260,000. More than one female participated in some of the spawns. Fertilization rates ranged from 0 to 95%, and hatch rates varied from 0.14 to 91.9%. The total number of eggs collected was 61,836,000. In the three ponds in which spawning took place, the number of spawns per female were 0.24, 1.5 and 2.2, indicating that females can spawn two or more times per season. In 1985, spawning also took place in the same ponds, however, the data on these spawns are not yet available. Although Lin's success is encouraging, the fish were 10 to 11-years-old and stocking densities were relatively low at one fish/25 m^2 in 56 of the spawns. Whether or not this success can be achieved with younger broodstock at higher densities must be examined.

4-6.2. SEAFDEC'S FLOATING CAGES

SEAFDEC reported a total of six natural spawns between April and August in the years 1980 through 1985 (Lacanilao and Marte, 1980; Marte et al., 1984a). The fish ranged in age from 3 1/2 to 7-years-old and, according to available information, ranged in size from 2.1 to 3.9 kg. Although fecundity was estimated at 600,000 from the 1980 spawns, no other fecundities were reported, nor were any fertilization or hatching rates available. This is presumably due to the problem of collecting eggs from floating cage spawns. Fewer than 3,000 eggs were collected from each of the two 1985 spawns, which probably represents less than 1% of the eggs that were spawned. The estimated number of spawns per female in 1980, assuming a 1:1 sex ratio among the 108 fish stocked in the cage, was 0.04. Until it is possible to achieve a greater frequency and consistency of spawning and an efficient means of collecting spawned eggs, the value of floating cages as a natural spawning facility is questionable.

4-7. INDUCED "SPONTANEOUS" SPAWNING

Whether maturation in females (i.e. completion of vitellogenesis in the largest class of maturing oocytes) occurs naturally or is induced by long photoperiod regimes or by hormones, completion is seldom followed spontaneously by final maturation and spawning. Hence, hormone treatments have been developed to induce final maturation in milkfish once the oocytes reach a minimum size of 660 μm. Unlike natural spawning, successful induced spawning depends on carefully monitoring the state of the gonad. There are five reported cases of hormone-induced final maturation that have been followed by spontaneous spawning (Lee et al., 1986a,c; Liao and Chen, 1984; Lin, 1982,1984; SEAFDEC, 1985). All other attempts have required stripping the ovulated eggs from the ovary for in vitro fertilization (ie. strip spawning, see Section 4-8).

Liao and Chen (1984) induced final maturation and spawning in a 5 to 6-year-old female with a body weight of 2.6 kg. The fish was injected with mullet pituitary (ADMP), puberon and vitamin E. Twenty-seven hours after injection, the female spawned. Although running males were present in the holding tank, the eggs were unfertilized.

In 1982, Lin induced final maturation and spawning in an 8 to 9-year-old female weighing 5 to 7 kg (Lin, 1982). Two injections were required with the first being a combination of HCG and mullet pituitary and the second, HCG alone. Thirty-six hours after the second injection, the female spawned 150,000 eggs. Although two running males were present in the holding tank, the eggs were not fertilized.

In 1983, Lin injected a 9 to 10-year-old female weighing 7.0 kg with HCG and placed her with two running males in a holding tank (Lin, 1984). Eight hours later, the female spawned and released 800,000 eggs, 55% of which were fertilized. Of these, 68.2% subsequently hatched.

SEAFDEC (1985) reported inducing final maturation and spawning using LHRH-a with the subsequent release of 700,000 eggs. No other information was reported.

In 1985, Lee et al. (1986a,c) carried out 50 attempts to induce final maturation and spawning with LHRH-a. Each attempt involved a single cholesterol pellet implant or IM liquid injection at a dosage of 200-250 μg. If the female showed no response, she was not reinjected. Thirty-four of the 50 attempts (68%) were successful. Nine (53%) of the 17 females that received an LHRH-a implant spawned while 19 (58%) of the 33 females that received an LHRH-a injection spawned within 20-26 hours following injection. All the females were 6 to 8-years-old and weighed between 3.15 and 5.2 kg. The first induced spawning took place on March 26, and the last spawning was on November 26. Only 11 of the 34 spawnings (32%) produced fertilized eggs, as a result of either poor egg quality, absence of males or from unknown causes. Fertilization rates ranged between 14 and 99.5% with hatch rates ranging between 8 and 79%. Fecundity estimates ranged from less than a hundred to 770,000.

Observations were made on an unfertilized and a fertilized spawn and, in both cases, two running males were present. In the unfertilized spawn, no courtship or spawning behavior by either the female or the males was observed, and the female slowly released 39,000 eggs over a period of 4 hours. In the fertilized spawn, both males participated, frequently chasing and nuzzling the vent of the female, and on several occasions, "sandwiching" the female tightly between them. Both the males and the female visibly shuddered when releasing gametes, which took place repeatedly during a 50 minute period. Fecundity, fertilization and hatching rates were estimated at 520,000, 94% and 18.2%, respectively.

These observations suggest that a running ripe condition is not a reliable criterion for predicting male participation in a spawn. Further, male spawning behavior may be important not only for fertilization, but for inducing egg release by the female.

4-8. INDUCED "STRIP" SPAWNING

Egg retention after induced final maturation and the release of eggs which are not subsequently fertilized are common occurrences that have necessitated sacrificing the female, stripping the ovaries, and fertilizing the eggs <u>in</u>

vitro. Until recently, this has been the most successful method of "spawning" both wild and pond-reared milkfish (Vanstone et al., 1977; Hsiao and Tseng, 1979; Kuo et al., 1979; Liao et al., 1979; Tseng and Hsiao, 1979; Lee and Weber, 1983; Juario et al., 1984). Lam (1984) provides most of the specific details concerning these attempts. In all cases, the oocytes were larger than 660 μm in diameter prior to hormone injection. The reported weights of females ranged between 3.5 and 10 kg. All of the attempts used intramuscular injections of SPH, CPH, ADMP and HCG, either alone or in combination. Between one and five injections were required, and the eggs were ready for stripping 6 to 24 hours after the first injection. A combination of 48.8 mg of CPH with 4,878 IU HCG/kg body weight, or 20 mg SPH with 3,000 IU HCG/kg body weight was the most productive treatment (Kuo et al., 1979; Juario et al., 1984).

After stripping, the eggs were fertilized in vitro using the dry method with milt stripped from running males (Juario and Duray, 1983). Fertilization rates resulting from these attempts have been relatively low, ranging between 0 and 59% (Juario et al., 1984).

4-9. SPERM PRESERVATION

One of the major problems with in vitro fertilization, as well as with induced spawning in milkfish, has been the unavailability of running ripe males. Several attempts have been made, therefore, to remove sperm from the males when it was available and to preserve and store it for later use. Lee and Kuo (unpub.) made a preliminary attempt to preserve milkfish sperm by keeping the sperm under 4^{o}C with or without extender. The fertilization rate of 1-week-old sperm without extender was 54%, compared to 48% and 52% with fresh sperm in salinities of 35ppt and 30ppt, respectively. Hara et al. (1982) tested different extenders for preserving sperm at near zero temperatures (0 to 4^{o}C) and in liquid nitrogen

(-196°C). After 4 to 5 days, cryopreserved milkfish sperm showed better fertilization capacity than fresh sperm and those preserved at near-zero temperatures. Milkfish serum proved to be the best extender.

Hara and Tiro (1983) found DMSO to be a better cryoprotectant than glycerine for milkfish sperm. Apparently, sperm preserved for 10 days in milkfish serum containing 12.5% DMSO yielded even higher fertilization and hatching rates, as well as a higher survival rate of larvae at 21 days, than fresh semen.

4-10. SUMMARY

It was pointed out earlier that the predominant problems confronting attempts to artificially propagate milkfish are the length of time these fish require to reach sexual maturity and secondly, the manifestation of a block to seasonal rematuration and spawning in sexually mature fish held in captivity. This chapter has focused on the work which has been carried out to overcome these two problems. The progress which has resulted from this work is summarized here.

4-10.1. SEXUAL MATURITY

Thus far, attempts to accelerate sexual maturity in milkfish have been unsuccessful. Some evidence suggests, however, that this may be achievable in males. Until a solution is found, mature milkfish must continue to be captured from the wild or immature milkfish must be maintained in captivity until they reach the age of 5 years or older. A weight of at least 3 kg may provide a rough indication of sexual maturity. Obtaining gamete samples during the reproductive season is, however, the only foolproof means of identifying potential broodstock.

4-10.2. SEASONAL REMATURATION AND SPAWNING

Various methods are now available to capture potential milkfish broodstock; the suitability of each depends on the depth and bottom profile of the particular habitat. Once captured, the fish can be transferred short distances in water-filled plastic bags, or long distances in environmentally controlled transport tanks. Injuries can be treated with antibiotics, and gonadal atresia resulting from stress to mature fish can be countered with hormones.

A high protein diet may be required for natural rematuration and natural spawning may also require salinities near normal seawater and temperatures above 24^{o}C. Even though broodstock of less than 7 years of age, stocked at high densities, have spawned naturally, consistent success has been achieved at only one facility using 10 to 11-year-old fish stocked at lower densities.

Rematuration in 7-year-old fish appears to have been accelerated under controlled photoperiods. The most dramatic success with inducing rematuration in fish of this age has been achieved with a hormone therapy. This therapy consists of 200 μg of LHRH-a and 250 μg of 17-MT in the form of chronic-release pellets being implanted simultaneously, prior to the reproductive season.

Younger broodstock have also been induced to spawn, however, gonadal maturation must be carefully monitored prior to the attempt. Fertilized "spontaneous" spawning has been induced with a single 10,000 IU injection of HCG as well as single 250 μg injections of LHRH-a. Additional research is required, however, to obtain a consistently successful protocol. Preliminary behavioral observations during these spawns suggest that inducing the appropriate spawning behavior in males may be of considerable importance when using this approach. Fertilized "strip" spawns have been obtained after females were given one or more injections of a variety of hormone preparations capable of inducing final maturation and ovulation. Cryopreservation techniques have recently been developed which allow storage of sperm for up to 10 days. In conjunction with "strip" spawns, eggs can be fertilized _in vitro_ using either fresh or preserved sperm, but this necessitates the sacrifice of valuable broodstock. Until a non-destructive stripping technique is developed, natural and induced spontaneous spawns are preferable.

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REFERENCES

Chaudhuri, H., J.V. Juario, J.H. Primavera, R. Samson, and R. Mateo. 1978. Observations on artificial fertilization of eggs and the embryonic and larval development of milkfish, _Chanos chanos_ (Forsskal). Aquaculture 13: 95-113.

Crear, D. 1980. Observations on the reproductive state of milkfish populations _Chanos chanos_ from hypersaline ponds on Christmas Island (Pacific Ocean). Proc. World Maricul. Soc. 11: 548-556.

Hara, S. and L.B. Tiro, Jr. 1983. The effect of cryoprotectant on the cryogenic preservation of the sperm of milkfish, _Chanos chanos_. Paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines, 4-8 October, 1983. p. 19.

Hara, S., J.T. Canto, Jr., and J.M.E. Almedras. 1982. A comparative study of various extenders for milkfish, _Chanos chanos_ (Forsskal), sperm preservation. Aquaculture 28: 339-346.

Hsiao, S.M. and T.C. Tseng. 1979. Induced spawning of pond-reared milkfish, _Chanos chanos_ Forsskal. China Fish. Monthly 330: 7-13 (in Chinese with English abstract).

Juario, J.V., G.F. Quinito, J.E. Banno, and M.R. Natividad. 1980. The effects of exogenous hormone injections on milt consistency in newly-caught wild mature milkfish, Kalikasan. Philippines J. Biol. 9: 321-326.

Juario, J.V. and M.N. Duray. 1983. A guide to induced spawning and larval rearing of milkfish, Chanos chanos (Forsskal). SEAFDEC Tech. Rep. 10 with IDRC. 22 pp.

Juario, J.V., M.N. Duray, V.M. Duray, J.F. Nacario, and J.M.E. Almendras. 1984. Induced breeding and larval rearing experiments with milkfish Chanos chanos (Forsskal), in Hawaii. Aquaculture 36: 61-70.

Kumagai, S. 1984. The ecological aspects of milkfish fry occurrence, particularly in the Philippines. In: J.V. Juario, R.P. Ferraris, L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc., Metro Manila, Philippines. pp. 53-68.

Kuo, C.-M. 1982. Progress on artificial propagation of milkfish. ICLARM Newsletter 5: 8-10.

Kuo, C.-M. 1984. Natural spawning of captive milkfish in Taiwan. ICLARM Newsletter 1984. pp. 18-19.

Kuo, C.-M., C.E. Nash, and W.D. Watanabe. 1979. Induced breeding experiments with milkfish, Chanos chanos (Forsskal), in the Philippines. Aquaculture 16: 247-252.

Lacanilao, F.L. and C.L. Marte. 1980. Sexual maturation of milkfish in floating cages. Asian Aquaculture 3: 4-6.

Lacanilao, F.L., C.L. Marte and T.J. Lam. 1984. Problems associated with hormonal induction of gonad development in milkfish (Chanos chanos Forsskal). Proceedings, 9th Int. Comp. Endocrin. Symp., Hong Kong, December, 1980 (in press).

Lam, T.J. 1982. Applications of endocrinology to fish culture. Can. J. Fish. Aquat. Sci. 39: 111-137.

Lam, T.J. 1984. Artificial propagation of milkfish: present status and problems. In: J.V. Juario, R.P. Ferraris, L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc., Metro Manila, Philippines. pp. 21-39.

Lee, C.-S. 1985. Environmental factors in milkfish reproduction. In: C.-S. Lee and I.C. Liao (Eds.) Reproduction and Culture of Milkfish. Oceanic Institute, Hawaii, and Tungkang Marine Laboratory, Taiwan. pp. 99-114.

Lee, C.-S. and G.M. Weber. 1983. Preliminary studies on the maturation of milkfish _Chanos chanos_ in an environmentally controlled system. Paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines, 4-8 October, 1983. p. 20.

Lee, C.-S., C.S. Tamaru, J.E. Banno, C.D. Kelley, A. Bocek and J.A. Wyban. 1986a. Induced maturation and spawning of milkfish, _Chanos chanos_ Forsskal, by hormone implantation. Aquaculture 52: 199-205.

Lee, C.-S., C.S. Tamaru, J.E. Banno and C.D. Kelley. 1986b. Influence of chronic administration of LHRH-analogue and/or 17α-methyltestosterone on maturation in milkfish, _Chanos chanos_. Aquaculture (in press).

Lee, C.-S., C.S. Tamaru, C.D. Kelley and J.E. Banno. 1986c. Induced spawning of milkfish, _Chanos chanos_, by a single application of LHRH-analogue. Aquaculture (in press).

Lee, C.-S., G.M. Weber and C.S. Tamaru. 1986d. Effect of orally-administered 17α-methyltestosterone on spermatogenesis in immature milkfish, _Chanos chanos_ Forsskal. J. Fish. Biol. (in press).

Lee, C.-S., C.S. Tamaru and G.M. Weber. Studies on the maturation and spawning of milkfish, Chanos chanos Forsskal, in a photoperiod-controlled room. J. World Maricul. Soc. (in press).

Liao, I.C. and T.I. Chen. 1979. Report on the induced maturation and ovulation of milkfish (Chanos chanos) reared in tanks. Proc. World Maricul. Soc 10: 317-331.

Liao, I.C. and T.I. Chen. 1984. Gonadal development and induced breeding of captive milkfish in Taiwan. In: J.V. Juario, R.P. Ferraris, L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc., Metro Manila, Philippines. pp. 41-51.

Liao, I.C., J.V. Juario, S. Kumagai, H. Nakajima, M. Natividad and P. Buri. 1979. On the induced spawning and larval rearing of milkfish, Chanos chanos (Forsskal). Aquaculture 18: 75-93.

Lin, L.T. 1982. Further success in induced spawning of pond-reared milkfish. China Fish. Monthly 357: 17-19 (in Chinese with English abstract).

Lin, L.T. 1984. Studies on the induced breeding of milkfish (Chanos chanos Forsskal) reared in ponds. China Fisheries No. 378: 3-29 (in Chinese).

Lin, L.T. 1985. My experience in artificial propagation of milkfish - studies on natural spawning of pond-reared broodstock. In: C.-S. Lee and I.C. Liao (Eds.) Reproduction and Culture of Milkfish. Oceanic Institute, Hawaii, and Tungkang Marine Laboratory, Taiwan, pp. 185-203.

Marte, C.L., F.J. Lacanilao and J.V. Jaurio. 1984a. Completion of the life cycle of milkfish, Chanos chanos (Forsskal) in captivity. Paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines, 4-8 October, 1983. p. 21.

Marte, C.L., G.F. Quinito, L. Ma. B. Garcia and F.J. Lacanilao. 1984b. A guide to the establishement and maintenance of milkfish broodstock. SEAFDEC Tech. Rep. 11. 36 pp.

SEAFDEC. 1985. Rematured milkfish spawns. Asian Aquaculture, Vol. 7(5). pp. 1-2.

Tseng, L.C. and S.M. Hsiao. 1979. First successful case of artificial propagation of pond-reared milkfish. China Fisheries 320: 9-10 (in Chinese).

Vanstone, W.E., L.B. Trio, Jr., A.C. Villaluz, D.C. Ramsingh, S. Kumagai, P.J. Duldoco, M.M.L. Barnes and C.E. Duenas. 1977. Breeding and larval rearing of milkfish Chanos chanos (Pisces: Chanidae). SEAFDEC Tech. Rep. 3: 3-17.

Wainwright, T. 1982. Milkfish fry seasonality on Tarawa, Kiribati, its relationship to fry seasons elsewhere, and to sea surface temperatures (SST). Aquaculture 26: 265-271.

5. LARVAE AND LARVAL CULTURE

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5-1. INTRODUCTION

The milkfish is an important subsistence food fish whose culture has been practiced for centuries in coastal ponds of the Philippines, Indonesia and Taiwan. Relatively little is known, however, about its early life history (i.e. from the time that gametes are released by adults at offshore spawning grounds, to the time that the young are captured as fry from coastal nurseries for stocking in ponds). This is because the precise locations of spawning are unknown and eggs and early larvae in plankton samples are scarce.

Available data on embryonic and larval development in milkfish have been collected by culturists developing rearing techniques. The relatively few successful spawnings achieved to date have not afforded much opportunity for experimental rearing of larvae. Progress has been impeded by the lack of a standardized and reliable method for induced breeding, which is in turn related to insufficient broodstock (Kuo, 1982).

The objective of this report is to review the present status of larval biology and culture of milkfish. Considering the limited availability of data in this area and recent developments in marine finfish larval-rearing methodology, a comprehensive approach was deemed appropriate. Hence, I have integrated information on other species potentially applicable to milkfish larval-rearing methodology.

5-2. MORPHOLOGY

5-2.1. DEFINITION OF LARVAE

The "larval" stage in fish generally includes the period from hatching until the adult form is assumed at metamorphosis (Blaxter, 1969). Newly-hatched fish are sometimes called "yolk-sac larvae" until the yolk is resorbed, then "larvae" until the completion of metamorphosis. The term "fry" is commonly used by milkfish culturists in reference to the late larval stage (10-16 mm TL) that appears in shore waters (Kumagai, 1984). The term is used in this context throughout this report. The development of laboratory-reared milkfish larvae past 21 days has not been fully described. Hence, morphological criteria signifying the completion of the larval stage have not been defined for milkfish as they have larval Mugilidae (see review by Nash and Shehadeh, 1980). Available information indicates that larvae enter the transition stage by 28 days, and attain the juvenile stage by 35 days (Villaluz et al., 1983).

For practical reasons, milkfish larvae must be reared in the hatchery only until they are able to withstand relocation to nursery ponds. Preliminary rearing trials have indicated that 21-day-old laboratory-reared milkfish larvae are robust enough for stocking in nursery ponds (Liao et al., 1979). Hence, in this report, the larval-rearing period for milkfish refers to the first 21 days following hatching.

5-5.2. LARVAL DEVELOPMENT

The first morphologic descriptions of the eggs and larvae of milkfish were made by Delsman (1926, 1929) on the basis of samples that he obtained during plankton tows in the Java Sea. Delsman's descriptions of larval development were reproduced many years later by Schuster (1960) in his synopsis of extant biological data on milkfish. No other detailed information on larval morphology was available until 1977, when successful artificial fertilization of milkfish eggs following hormonal inducement of adults was achieved by Vanstone et al. (1977) and Chaudhuri et al. (1978). Detailed descriptions of early embryonic and larval development were provided by these workers.

The first complete continuous record of development of milkfish larvae to the fry stage was subsequently made by Liao et al. (1979). Important developmental features are summarized as follows: Fertilized eggs of the milkfish range from 1.10 to 1.25 mm in diameter. Newly-hatched larvae range from 3.2 to 5.3 mm in total length and possess a large yolk sac about 2.20 mm long and 0.28 mm wide. The larva is transparent with unpigmented eyes and no fin buds. The mouth is not formed and the anus is closed. Thirty-four to 35 preanal myotomes are observed. Resorption of yolk and concomitant increase in larval length are rapid within the first 24 hours after hatching. The anus and mouth are opened at 48 and 54 hours, respectively. By day 3, the yolk is completely absorbed and the eyes are fully pigmented. Feeding behavior is conspicuous at this time. Growth accelerates on day 8, and after 13 days the largest larvae measure about 10 mm in total length and are considered fry, although they are still too weak for stocking in ponds. The 18 to 20-day-old larvae measure 10.3-14.9 mm in total length, similar to that of wild fry. Their pigmentation pattern, in contrast, is more pronounced and their body weight is approximately twice that of wild fry of similar length. After 21 days, larvae measure 13.5-16.5 mm in total length, still within the range of wild fry. They appear more developed than wild fry of similar length as evidenced by the formation of pelvic fins 5-10 days before those of wild fry. The 21-day-old larvae are resistant to handling and are suitable for stocking in ponds.

Lam et al. (1985) reported that treatment of milkfish post-yolk-sac larvae with L-thyroxine-sodium (Eltroxin, Glaxo) by immersion in 0.5 ppm solution markedly accelerated their growth and development to the juvenile stage. After 15 days of treatment, larvae were silvery, opaque and adult-like in form, whereas control larvae were slender, transparent and incompletely silvered. These workers suggested that thyroxine may be used to shorten the nursery period of milkfish larvae. Incorporating of thyroxine (T_4) or 3,5,3'-triiodothyronine (T_3) in larval food was suggested as a solution to the practical problem of treatment by large-scale immersion. Further studies are needed.

5-3. BEHAVIOR

The behavior of laboratory-reared milkfish larvae has been described by Liao et al. (1979). Newly-hatched larvae are immediately active and exhibit a characteristic swimming behavior (Chaudhuri et al., 1978; Liao et al., 1979). The larva remains suspended head down in the water column near the surface, then sinks slowly in an oblique position with the ventral side oriented toward the surface. Upon reaching a depth of about 5-8 cm, the larva makes a sudden upward turn of 360° before swimming rapidly to the surface. This pattern of movement is often repeated during the first 2 days.

Chaudhuri et al. (1978) observed fast initial growth for 2 days concomitant to steady resorption of yolk. There was no further growth, however, from the 3rd to 6th day, when total mortality occurred. This critical period of high mortality coincided with the complete resorption of yolk and full formation of mouth and intestine. Liao et al. (1979) similarly reported a marked reduction in growth between days 3 and 6 when the larvae began a critical period of high mortality lasting for 2-3 days. Juario et al. (1984), summarizing results of larval-rearing experiments on milkfish from 1978-1981, also distinguished a critical period of generally high mortality beginning on day 4, corresponding to the onset of feeding, and lasting to day 7. Reasons for mortality during this critical period have not yet been identified, but the temporal association between the depletion of the yolk sac and the onset of feeding suggests that starvation is causal.

From day 3 to day 13, milkfish larvae show phototaxis and rheotaxis during the day, drifting passively with the currents at night. By day 10, larvae swim in a school and show strong rheotaxis during the day. Newly-caught milkfish fry similarly show strong rheotaxis in containers. Kawamura (1984) suggested that this behavior may be controlled by vision and aided by free neuromasts which are already well developed in fry. He also noted that newly-caught fry exhibit strong positive phototaxis, which weakens as they approach metamorphosis. Fry have sensitive eyes and are repelled by light stronger than 200-300 W in experimental tanks.

After day 14, the larvae swim more swiftly and circularly during the day and slower at night to counter the current. The larvae also exhibit strong phototaxis at night.

After day 18, the larvae are no longer sensitive to direct exposure to sunlight and they prefer to feed on algae growing on the tank wall.

The feeding behavior of wild milkfish fry has been described by Kawamura and Hara (1980). Upon sighting its prey, the larva positions its head so that the prey is perpendicular to its snout. The larva then assumes an S-flex or W-flex feeding posture before striking at its prey.

5-4. NUTRITION

The food and feeding habits of milkfish fry in natural habitats have been studied by a number of workers (Tampi, 1958; Schuster, 1960; Vicencio, 1977; Buri, 1980; Banno, 1983) and are reviewed by Santiago (see Chapter 7). In general, gut content analyses have revealed the presence of benthic, epiphytic and planktonic organisms, consisting largely of diatoms and lesser amounts of blue-green algae and zooplankton, and detritus. Results of one study (Banno, 1983), however, suggest that detritus is the primary source of food for wild fry.

5-4.1. EMPIRICAL FEEDS DEVELOPMENT

Initial attempts to rear larvae to metamorphosis following successful artificial fertilization of milkfish eggs had limited success (Vanstone et al., 1977; Chaudhuri et al., 1978; Liao et al., 1979).

Liao et al. (1979) successfully reared a total of 2,859 milkfish larvae to the fry stage (21 days) with survival rates ranging from 8.8 to 46.8% in different experimental groups. The larvae were distributed among two large 1.25 m diameter tanks and eight small 1 m diameter tanks. Rearing was conducted in seawater (34 ppt) at temperatures of 27.1 to 29.5°C. The pH ranged from 8.1-8.3. *Chlorella* sp. was added to all tanks starting on day 1 and was maintained at densities between 50 and 350 X 10^4 cells/liter thereafter. On day 2, either a combination of fertilized eggs and larvae of oysters and rotifers *Brachionus plicatilis* (80-200 μm) or rotifers alone was provided. Larvae fed a combination of oyster eggs and larvae (30-300 organisms/ml) and rotifers (10-200 organisms/ml) for the first six days generally exhibited higher survival than those fed only rotifers. Groups of 14-day-old larvae or older were given wild copepods including Cyclops sp. (200-600 µm), cultured copepods Tigriopus japonicus (120-1,500 µm) and brine shrimp nauplii Artemia salina (160-1,000 µm), as well as flour and prepared feed. The effect of these foods on survival was indeterminate.

Juario et al. (1984) recently summarized results of larval-rearing experiments on milkfish conducted from 1978 to 1981. Larvae were reared in 600 liter circular fiberglass tanks at temperatures of 26-30^{o}C and at a salinity of 34 ppt. In 1978 and 1979, survival rates in various rearing tanks ranged from 3-30% when oyster trochophores and Chlorella virginica-fed rotifers were fed to first-feeding larvae. In 1980 and 1981, survival rates ranged from 8-71% when the phytoplankters Isochrysis galbana and Tetraselmis chuii were introduced together with Chlorella sp. and Chlorella sp.-fed rotifers, despite the elimination of oyster trochophores. The feeding schedule developed by these workers is shown in Fig. 1. In addition to several species of phytoplankton and rotifers, newly-hatched Artemia salina was provided beginning on day 10. Copepodites and adults of Tisbintra elongata were also included in this feeding schedule, although no data concerning their use were provided. Inclusion of artificial feeds beginning on day 14 (Fig. 1) is based on the results of a recent study in which hatchery-bred milkfish larvae were successfully weaned from live to artificial foods (Duray and Bagarinao, 1984). The highest survival rates achieved to date under laboratory conditions were recorded in various experimental tanks using this mixed dietary regime. However, in view of the large differences in survival rates between groups under supposedly identical rearing conditions, the

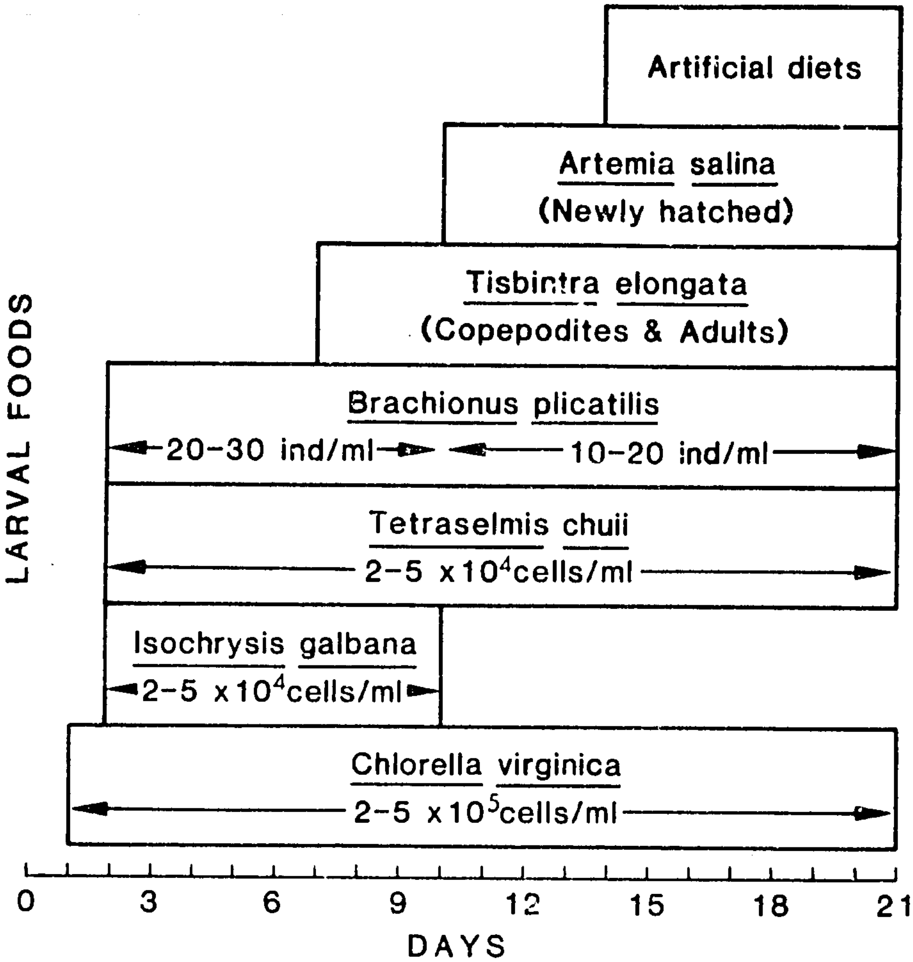

Fig. 1. Feeding schedule for milkfish larvae during the 21-day rearing period (after Juario et al., 1984). Inclusion of artificial diets is based on results of early weaning studies by Duray and Bagarinao (1985).

variations in initial stocking densities, and the suggestion that viability of larvae was related to the method of fertilization (artificial or natural), it is difficult to definitively attribute high survival to any specific factor(s) associated with these trials.

Recently, a commercial milkfish farmer in Taiwan reported successful rearing from naturally-spawned eggs of approximately 4.4 X 10^6 milkfish fry (10-16 mm TL) in outdoor, earthen-bottom ponds (150-3,000 m^2) with an average survival of approximately 17.7% and highest survival of about 76.2% (Lin, 1985). Unfortunately, feeding regimes were not specified in this report. According to Kuo (1984), who summarized the larval-rearing methods used by Lin (1984), food organisms, including algae (Chlorella sp.) and rotifers, were established in rearing ponds before larvae were stocked. Fertilized oyster eggs and egg yolk were provided from 72 hours onward, and Chlorella sp., rotifers, formulated eel feed, and wheat flour were provided during the late stages (10-22 days posthatching) of rearing. Again, the importance of these various feeds toward the success of these trials cannot be assessed o the basis of available data. Considering the rearing conditions used, it is likely that the larvae derived a significant portion of their food requirements from incidental planktonic organisms present in these large, outdoor ponds.

5-4.2. ROLE OF PHYTOPLANKTON

Juario et al. (1984) observed that although milkfish larvae ingested *Isochrysis*, *Tetraselmis* and *Chlorella* spp., there was no evidence that the larvae could digest them, and intact *Chlorella* were observed in the posterior region of the gut near the anus. Recent studies, however, have provided evidence for direct utilization of certain algal cells by milkfish larvae and for qualitative differences between algal types.

Ultrastructural characteristics of milkfish hepatocytes have been determined to be diagnostic of quality and quantity of feed or the general nutritional condition of the fish (Storch and Juario, 1983; Storch et al., 1983, 1984). Juario and Storch (1984) observed complete mortality in *Chlorella*-fed fry after 6 days feeding. Hepatocyte ultrastructure of *Chlorella*-fed fry corresponded to that of starved fry, indicating that milkfish fry could not directly utilize *Chlorella*, which has a rigid cell wall. *Tetraselmis*-fed fry also died after 6 days feeding, although hepatocytes showed evidence of recovery from a starved condition before showing degenerative changes. Although the fry could directly utilize *Tetraselmis*, the alga was nutritionally inadequate for growth and survival. In contrast, *Isochrysis*-fed fry survived until the experiment was terminated at day 35, although growth was poor. Hepatocytes of *Isochrysis*-fed fry at day 35 showed degenerative changes, which became more pronounced as the rearing period progressed. These results indicate that *Isochrysis* was also directly utilized by milkfish fry. *Isochrysis* was superior to *Tetraselmis* as food, although it was nutritionally inadequate for growth of fry if used alone. Hence, improved larval survival associated with the introduction of *Tetraselmis* and *Isochrysis* to rearing tanks may be partly attributed to the direct nutritional benefit that these algae provide to the larvae.

Pantastico et al. (1983) studied the effects of feeding different species of freshwater algae to milkfish fry (2.54 mg). The blue-green alga, *Oscillatoria quadripunctulata*, alone or in combination with *Chroococcus dispersus*, resulted in high growth and survival compared to the diatom *Navicula* sp. alone. Studies with ^{14}C-labeled algae revealed high assimilation rates in fry fed with *Oscillatoria* sp. or *Chroococcus* sp. alone, whereas negligible amounts of *Navicula*, *Chlorella*, and *Euglena* spp. were assimilated.

Villegas et al. (1983) studied the effects of feeding rotifers reared on different algal diets (*Chlorella* sp., *Isochrysis galbana* and *Tetraselmis tetrahele*) to wild milkfish fry (13 mm mean length) for 30 days. Fastest growth was obtained in fry fed rotifers reared on *Tetraselmis* sp., while poorest growth was obtained in fry fed rotifers reared on *Chlorella* sp.. Acosta and Juario (1983) reported that milkfish fry given rotifers feeding on a mixture of *Chlorella* sp., *Isochrysis galbana*, and *Tetraselmis* sp. exhibited significantly higher growth than those fed rotifers reared on a single species of algae. Growth of fry given rotifers feeding on *Tetraselmis* sp. or *Isochrysis* sp. was similar, while poorest growth was obtained in fry fed rotifers reared on *Chlorella* sp.

Segner et al. (1984) studied the ultrastructural alterations in hepatocytes of milkfish fry after feeding them rotifers grown on unialgal cultures of *Isochrysis galbana*, *Tetraselmis* sp. and *Chlorella* sp. Distinct differences in the nutritional value of these rotifers were evident. Best results were obtained from fry reared on *Isochrysis* sp.-fed rotifers, and poorest results were obtained from fry reared on *Chlorella* sp.-fed rotifers. None of the three rotifer diets, however, produced an hepatocyte ultrastructure comparable to that observed in fry reared on a mixture of artificial feed and newly-hatched *Artemia* nauplii.

5-4.3. PRODUCTION OF LIVE FOODS

The logistics of producing live foods daily in a commercial marine finfish hatchery are considerable. Production cycles of planktonic food organisms must be synchronized to larval requirements. This often entails maintaining series of culture tanks of each organism in order to have adequate stocks of live food throughout the larval-rearing period. Limitations to larval stocking densities imply that commercial production will require the use of large culture systems. Large systems impose heavy demands on live foods in order to maintain optimal concentrations for feeding and to replace those lost through water exchange. Costs can become prohibitive when several different kinds of food organisms are required, as with presently recommended feeding schedules for larval milkfish (Juario and Duray, 1983; Juario et al., 1984). Methods which reduce or eliminate the need for live foods or increase the efficiency of their use are of immediate importance and may include the following: 1) increasing the efficiency of live food production, 2) improving the nutritional value of food organisms, 3) increasing the convenience of the use of live foods through storage, 4) using live food in combination with artificial feeds, 5) early weaning to artificial foods, and 6) developing artificial diets that can be used from first-feeding.

Although oyster eggs and larvae are frequently used during experimental rearing of milkfish larvae (Chaudhuri et al., 1978; Liao et al., 1979; Juario et al., 1984), results suggest they have marginal nutritional value. Juario and Natividad (1980) noted that the introduction of oyster trochophores into milkfish larval-rearing tanks hastened the deterioration of water quality. The preparation of these feeds is also laborious and time consuming (Liao, 1975; Howell, 1979). Their utilization during larval rearing of milkfish does not appear to merit further evaluation.

At present, mass production of the rotifer Brachionus plicatilis is essential for rearing marine fish larvae. Due to the high requirement for these organisms, the mass production of fish fry is limited by their availability. For a discussion of rotifer nutrition, see Kitajima et al. (1979), Fukusho (1983), and Watanabe et al. (1983). It may be tentatively concluded that, for milkfish larvae, the dietary value of yeast-fed rotifers may be improved by enrichment with

Tetraselmis sp. or Isochrysis sp. rather than Chlorella sp.

Nauplier through adult stages of copepods are considered to be an important natural food for larval marine fish (May, 1970; Houde, 1972; Hunter, 1980) and are known to possess high nutritional value (Watanabe et al., 1983). In Japan, mass culture techniques have been established for the copepod, Tigriopus japonicus, the species most commonly used for rearing marine fish larvae (Fukusho, 1980; Fukusho et al., 1980, Kuronuma and Fukusho, 1984). The nutritional value of T. japonicus may also be improved by culturing it with w-yeast rather than baker's yeast (Fukusho et al., 1980). The use of copepods for larval rearing of milkfish has not been carefully evaluated. In view of their high dietary value, the inclusion of these organisms in the diet will probably be beneficial.

Nauplii of Artemia salina are often important during the latter stages of rearing marine fish larvae when larger food organisms are required.

The value of these various plankton-enrichment techniques must be evaluated. Since these techniques are easily used and the potential benefits great, they merit detailed investigation. The use of rotifers and Artemia in frozen or freeze-dried form also merits evaluation.

Optimal concentrations of food organisms to be used during rearing will vary with species, age of fish cultured and the type of food organism used, as well as other factors such as temperature and light (Houde, 1972). It is important to control food density in order to maximize opportunities for feeding and to prevent overfeeding. Many larvae appear to lack satiation mechanisms (Hunter, 1980). They continue to feed even after the digestive tract is full, and then excrete live or partially digested food organisms (Kuo et al., 1973; Spectorova et al., 1974). A knowledge of optimal food densities also permits judicious use of limited food supplies. Studies on optimal food concentrations for larval rearing of milkfish are needed.

High stocking densities of larvae are commercially advantageous but lead to poor survival and growth (Houde, 1972; Nash and Shehadeh, 1980). Nash and Shehadeh (1980) recommended a stocking density of 20 larvae per liter for grey mullet in 4,800 liter indoor or 14,000 liter outdoor rearing tanks. At 25% projected survival at day 50, a maximum density of 5 juveniles per liter is used. They recommended that higher initial stocking densities (to about 50 larvae per liter) be used if lower survival is anticipated. Juario and Duray (1983) indicated a stocking density of about 5-10 larvae per liter for rearing milkfish in 600 liter fiberglass tanks. Studies are needed to determine optimum stocking densities during larval rearing of milkfish. These will vary with rearing conditions employed.

5-4.4. ARTIFICIAL FOODS

The use of artificial feeds prepared from either synthetic or naturally occurring components for larval rearing of milkfish has received relatively little attention. Flour and prepared feed of undefined composition were used during initial larval-rearing experiments with milkfish (Liao et al., 1979), although the effect of their use could not be determined. The use of prepared feed was recommended by Juario and Duray (1983), beginning on day 10 of the 21-day larval-rearing period, although details for its use were not provided.

A fundamental problem impeding progress in the area of artificial feeds development is an inadequate knowledge of larval nutrition. Assessing nutritional requirements in the larval stages is extremely difficult due to larvae size and vulnerability (van Limborgh, 1979). Related problems include acceptability of non-living feeds, continuous distribution of such feeds, and the maintenance of tank hygiene.

In the absence of data on nutritional requirements of larvae, it has been suggested that dry matter composition of natural prey be used as a basis for formulating artificial diets (van Limborgh, 1979). An objection to this approach is that it is predicated on knowledge of what constitutes a complete natural diet, which in fact may be quite diverse. The composition of milkfish egg yolk, which presumably has an ideal balance of constituents for early larvae and which may be easily analyzed, should provide a better basis for formulating an artificial diet. Once yolk composition is established, feed experiments may be conducted in which constituents are varied around their mean yolk values. The possibility that egg yolk composition (i.e., egg quality) is influenced by diet of the maternal spawner or other conditions of broodstock keeping (e.g., space, temperature) should not be ignored.

It has also been suggested that nutrient requirements of bigger fish be used as a basis for developing larval diets (van Limborgh, 1979). Recent studies have demonstrated the feasibility of rearing wild milkfish fry and juveniles on semi-purified formulated dry diets. Santiago et al. (1983) determined that formulated dry diets containing 40% crude protein (from fish meal) were adequate for milkfish fry (mean total length, weight = 13 mm, 15 mg) reared in freshwater. Mean weight gains of 0.16-0.18 g and survival rates of 83-95% were obtained after 5 weeks of feeding. Camacho (1979) reported that a formulated diet containing 50% crude protein (from 55% casein and 14% gelatin) produced 90% survival and 0.15 g weight gain after 28 days in wild milkfish fry reared in brackishwater. Lim et al. (1979) determined a dietary protein level of 40% (from casein) to be optimal for growth and survival of wild milkfish fry (40 mg average weight) fed 10% biomass per day in a controlled seawater environment. A mean weight gain of 0.135 g and survival of 30% were observed after 30 days. Growth and survival rates were lowered when dietary protein levels exceeded 40%. Teshima et al. (1984) reported that milkfish juveniles (82-510 mg body weight, 20-32 mm body length) require a dietary protein level of approximately 50% fed at 30-50% body weight per day, for optimum growth in brackishwater or seawater aquaria. Protein requirements varied depending upon the quality of the protein source.

The successful use of artificial feeds for rearing milkfish fry and juveniles suggests the feasibility of applying such feeds at even earlier stages of development, either in combination with live foods or through early weaning. Successful weaning of 2-week-old hatchery-bred milkfish larvae from live foods to artificial diets was recently reported (Duray and Bagarinao, 1984). Larvae were weaned abruptly from a rotifer diet to various artificial diets, including commercial larval feed, artificial plankton, experimental milkfish fry diets, and moist egg diet. On day 43 (4 weeks after weaning), survival rates of larvae fed artificial diets (38-63%) were comparable to those obtained in larvae fed <u>Artemia</u> nauplii (42%). However, mean body weight was highest in larvae fed <u>Artemia</u>. These workers suggested that weaning milkfish larvae to artificial diets as early as one week from hatching may be possible.

Storch et al. (1983) compared hepatocytes of milkfish fry fed various artificial diets (carbohydrate-, lipid-, protein-oriented) or live foods (<u>Artemia</u> sp., <u>Brachionus plicatilis</u>) and found that only live foods resulted in a quick reestablishment of hepatocyte ultrastructure from a starved condition. Among the artificial diets offered, the protein-oriented (50% protein from casein) resulted in the best recovery of hepatocyte ultrastructure, with glycogen levels surpassing those observed in wild fry. The lipid-oriented diet resulted in increased deposition of small lipid droplets in the cytoplasm and nuclei of hepatocytes, possibly indicating the beginning of a lipoid liver disease.

For practical purposes, the effectiveness of a larval milkfish diet must be judged on the basis of its ability to support growth and survival up to fry stage. The measurement of growth and other parameters (e.g., food intake or wastage, digestibility, and N-retention) are problematic in the larval stages (van Limborgh, 1979). Histological techniques, which are readily applied to the larval stages, may provide valuable supplementary information to growth and survival studies, and may be particularly useful in making fine distinctions between similar diets. Another advantage of the histological approach is that ultrastructural changes in liver and other internal organs reflecting the effect of a diet are detected relatively early (Storch and Juario, 1983; Storch et al., 1983, 1984). The utility of the histological approach appears at present to be limited by a lack of information on the relationships between short-term dietary effects on internal organ ultrastructure and long-term dietary effects on growth and survival.

Ferraris et al. (1983) studied the developmental morphology of the digestive tract in hatchery, pond, and cage-reared milkfish from the yolk-sac-larval through adult stages. The digestive tract of the newly-hatched larvae is a simple undifferentiated tube. After 3 days, the esophagus and intestine are distinguishable. During the late larval (fry) stage, the stomach differentiates into cardiac and pyloric regions, while goblet cells develop in the intestine. At metamorphosis (39 days), the cardiac stomach apparently becomes functional with formation of pepsin-secreting glands. The structural complexity of the digestive tract increases with further growth. Further studies are required to elucidate the relationships between the morphological and functional development of the digestive tract. Such information will be important in the development of appropriate diets for different stages. The concept of autodigestion of food in the gut of larval fishes, in which foods such as Artemia or whole mussel tissues serve not only as a source of food, but also of digestive enzymes, is relevant to the development of appropriate diets for the early larval stages where exocrine glands that secrete digestive juices are not yet developed (Appelbaum, 1985).

5-4.5. EXTENSIVE LARVAL CULTURE

Extensive larval milkfish culture using fertilizer-induced zooplankton blooms has not been studied in detail. Such a system uses an undefined, heterogeneous feed regime, offering a wide variety of food types and sizes. Thus, acceptable food materials are always available for the larvae as they grow and feed requirements are altered. A disadvantage is that extensive culture systems are difficult to manage and replicate. However, in the absence of knowledge of the optimal nutritional requirements of milkfish larvae and how to meet them, extensive culture may be the preferred approach from an economic standpoint. As described earlier, Vanstone et al., (1977) obtained relatively good survival of milkfish larvae in an unmanaged 4 m diameter tank in which a bloom of Chlorella spp., diatoms, protozoa, Enteromorpha, and mixed copepods occurred. Large-scale production of milkfish fry from naturally-spawned eggs in large outdoor ponds with simple (i.e., semi-intensive) management has been demonstrated in Taiwan (Lin, 1984, 1985).

Carreon et al. (1984) reported good growth and survival in milkfish fry fed natural plankton in freshwater recirculating systems. Plankton was produced by fertilizing impounded tap water with chicken manure and complete fertilizer. They hypothesized that the fry derived nutritional benefit from a high load of non-pathogenic bacteria and microscopic organic (cellular or non-cellular) matter in suspension by a process known as "leptopel" feeding (Morris, 1955). According to this concept, microscopic organic particles are ingested through the aid of mucous secretions and ciliary activity of epidermal cells in the buccal cavity.

Kafuku and Kuwatani (1976) observed that in milkfish, the epibranchial organ (elongated diverticula of the pharynx located behind the gill cavity) appeared before any other parts of the digestive system, being rudimentary in as early as 14 mm fry and completely developed in 19 mm fry. Observations made by these workers supported the view that the organ functioned as part of the digestive system, probably involved in concentration and direction of food from the pharynx to the esophagus (Kuwatani and Kafuku, 1978). They suggest this organ takes a role in the utilization of microscopic organic matter in association with "leptopel" feeding. Experimental evidence is required before the importance of this phenomenon to the larval culturist can be accurately assessed.

5-4.6. FOOD SELECTION

Knowledge of the development of sense organs and the criteria used by larvae in food selection is important for the establishment of effective feeding methods during larval rearing. Vision is the most important sense for milkfish fry in feeding (Kawamura, 1984). Newly caught fry exhibit a well-developed regionally differentiated duplex retina. Although rod density is low at this stage, retinomotor response is observed, indicating that dark and light adaptation is possible. A retinal tapetum lucidum is present in the pigment epithelium which may be functional under subdued light conditions -- as in turbid shore waters. The importance of vision to the fry is indicated by their inability to feed in the dark (Kawamura and Hara, 1980). In contrast, juveniles are able to feed in the dark, although less efficiently than in lighted conditions. This ability is attributed to development of the chemical and auditory senses. Evidence suggests that juveniles possess color vision (Kawamura and Nishimura, 1980), although this aspect has not been studied in fry.

A diurnal variation in feeding pattern was observed in laboratory-reared milkfish larvae (9-21 days post-hatching) under natural light conditions (Hara et al., 1983). Active feeding commenced at 0630 h (1560 lux) for day 9 larvae, 0540 h (131 lux) for day 15 larvae, and 0500 h (0.08 lux) for day 21 larvae, suggesting that older larvae were able to begin feeding under lower light levels than younger larvae. Feeding decreased during the evening and ceased at 2300 h for day 9 and day 15 larvae, although day 21 larvae still had food in the gut at 0100-0200 h.

Optimal light regimes and intensities for rearing milkfish larvae have not been determined. Blaxter (1980) suggested that studies determining the optimum light intensity, color, distribution and daylength may be rewarding considering the important role that vision plays in feeding of marine fish larvae.

Teshima et al. (1984) found that growth of milkfish juveniles on artificial diets was significantly affected by particle size of diets. Studies determining the effect of food size on feeding response and growth in milkfish larvae are required.

Mechanoreceptors and chemoreceptors may also play a role in food selection by larvae (Iwai, 1980). Newly-caught milkfish fry have numerous free neuromasts with well developed cupulae on the head and a few on the trunk (Kawamura, 1984).

Since development of taste buds in the oropharyngeal cavity of marine fish larvae is delayed until after the onset of feeding, the gustatory sense does not appear to be important in food selection during the early stages (Iwai, 1980). However, taste buds are well developed and may be involved in the secondary selection of food within the mouth during late larval stages. Newly-caught milkfish fry have numerous taste buds in the epithelium of the oral cavity and gill arches and in the epidermis of the upper and lower lips (Kawamura, 1984). Taste buds increase in number and size with growth. Villaluz and Unggui (1983) observed that wild milkfish fry fed an artificial diet and Brachionus sp. occasionally rejected food particles taken into the mouth, indicating the secondary selection of food within the mouth. Large food particles were ignored, indicating the importance of size in food selection.

Olfaction may also play a role in food detection in fish larvae (Iwai, 1980). Kawamura (1984) observed that the division of incurrent and excurrent nares of the olfactory organ was incomplete in about one-half of the newly-caught milkfish fry that he examined. Division of nares was completed within 5 days after capture, thus making possible the separation of incoming and outgoing water. Although the olfactory epithelium was not lamellated in newly-caught fry, lamellation increased with growth.

The proposed role of olfactory and gustatory chemoreceptors enhancing feeding by larval food selection suggests the possibility of incorporating appropriate chemical agents into artificial diets for olfactory and gustatory enhancement of feeding (Atema, 1980; Iwai, 1980). This possibility is particularly attractive since chemical food signals are effective in small amounts and may be readily available as by-products of food processing (Atema, 1980).

Newly-caught milkfish fry have well-developed semicircular canals, but not well-differentiated pockets (Kawamura, 1984). The presence of neuromast cells with cupulae and otoliths in the main and anterior pockets indicates that the inner ear can function in hearing and equilibrium maintenance. Fujiya et al. (1980) reported that red sea bream

(*Pagrus major*) as small as 20 mm long could be trained to gather around an underwater speaker in response to sound pulses in experimental tanks before food was given. Acoustic control of larval feeding behavior may have applications for use with artificial diets dispensed from automatic feeders.

5-5. ENVIRONMENTAL CONDITIONS FOR REARING

Little information exists on the optimum temperature, salinity and water quality requirements for milkfish during larval development. Villaluz and Unggui (1983) determined that growth and development of wild milkfish fry were faster at high temperatures (25.8-35.2°C) than at ambient (23.7-28.9°C) or low (17.5-22.6°C) temperatures when maintained in glass aquaria at 20 ppt salinity. Survival was similar at high (99.7%) and ambient (97.7%) temperatures but relatively poorer at low (76.7%) temperatures. Low temperatures (<22.6°C) and hypoxial conditions (<1 ppm O_2) decreased activity and food intake, inhibiting growth and development at the juvenile stage. At low temperatures, dead fish exhibited scoliosis and lordosis--symptoms of a vitamin C deficiency--suggesting that diet conversion efficiency, as well as food intake, was lowered. These workers concluded that the approximate temperature limits for successful development of wild fry up to the juvenile stage are 23.7 to 33°C.

In some fish species, optimum rearing temperature varies with age. The effects of temperature on survival and growth of milkfish during early larval development have not been studied.

Holliday (1969) concluded that survival and growth of larvae could be improved by rearing at lowered salinities (10-16 ppt) because these levels were iso-osmotic with body fluids, reducing the energy costs of osmoregulation.

Optimal rearing salinities for milkfish larvae have not been determined. Salinity tolerance studies indicate that hatchery-bred milkfish larvae are moderately euryhaline on day 0 (8-37 ppt) and day 14 (6-28 ppt), highly euryhaline on day 21 (0-70 ppt), and stenohaline (27-28 ppt) on day 7 (Duenas and Young, 1984). This suggests that larval survival may be improved by rearing at a constant salinity of 27-28 ppt. Juario and Duray (1983) suggested that salinity in milkfish rearing tanks be gradually lowered to 28 ppt after day 5. More detailed studies in this area are required. A knowledge of salinity tolerance limits, salinity optima and osmoregulatory ability of milkfish larvae at various stages of development will provide a basis for selecting the optimal time and rate of salinity adjustment.

Oxygen requirements of milkfish larvae have not been investigated in detail. Juario and Duray (1983) indicated dissolved oxygen concentrations between 5-6 ppm were suitable for larval rearing of milkfish. Villaluz and Unggui (1983) observed that wild fry maintained in aquaria at temperatures ranging from 23.7-35.2°C continued to feed throughout the day if dissolved oxygen levels were above 1 ppm. Since oxygen supersaturation can sometimes occur in intensive culture systems, possible deleterious effects of high oxygen levels (e.g., "gas bubble disease") should also be investigated. Mortality associated with the presence of gas bubbles in the abdominal cavity or within intestinal organs during rearing of milkfish fry (>21 days) in fiberglass and concrete tanks has been reported (Lio-Po et al., 1984).

Thorpe and Wankowski (1979) discussed behavioral characteristics (e.g., rheostaxis, thigmotaxis, territoriality, response to moving particles) in juvenile Atlantic salmon (Salmo salar L.) in relation to rearing tank design. Radial flow tanks meet the behavioral requirements of these fish with respect to current velocities, manner of food particle presentation and distribution. Carreon et al. (1984) hypothesized that smooth water currents at tolerable velocities are essential for minimizing energy expenditure by larval fishes in feeding, swimming and other physiological activities, especially in species exhibiting rheotaxis, such as milkfish. They observed that at current speeds of 5 to 10 cm/s, milkfish fry in culture swam easily against the current and fed more frequently when they encountered food particles. Fry were observed to maintain positions within the raceways and intercepted food particles in eddies produced by the currents. Independent estimates have shown that the swimming speed of milkfish fry during sustained swimming falls within a narrow range of 9-11 cm/s (Kawamura, 1984). Studies on rearing tank designs incorporating features which meet the behavioral requirements of milkfish larvae are needed.

5-6. SUMMARY

Newly-hatched milkfish larvae range from 3.2 to 5.3 mm in total length and possess a large yolk sac. A critical period of high mortality, beginning on day 4 and lasting till day 7, is associated with yolk sac depletion and the onset of feeding. After 21 days, larvae measure 13.5-16.5 mm in total length, but appear more advanced in development than wild fry of similar length. The 21-day-old larvae are robust enough for stocking in nursery ponds. An understanding of the morphological, biochemical, and behavioral differences between reared and wild larvae is important for control of larval quality during culture.

The possibility of incorporating thyroxine (T_4) or 3,5,3'-triiodothyronine (T_3) in larval food to accelerate growth and development should be studied.

Milkfish larvae at the yolk-sac stage exhibit a swimming behavior characteristic of yolk-sac larvae of many marine species. The behavior probably maintains larvae at a constant depth in the sea and is energetically advantageous. From day 3 to day 13, milkfish larvae exhibit phototaxis and rheotaxis during the day, drifting passively with the currents at night. Milkfish larvae exhibit a sinuous feeding posture before striking at prey, a behavior typical of larval clupeoid fishes.

In natural habitats, milkfish fry feed on various planktonic organisms, including diatoms, blue-green algae and zooplankton, as well as detritus. Although high survival rates have occasionally been achieved under laboratory conditions using a mixed dietary regime including several species of phytoplankton (i.e., Chlorella virginica, Tetraselmis chuii, and Isochrysis galbana), rotifers, and Artemia salina, optimal feeding regimes have not been established.

Milkfish fry cannot directly utilize Chlorella sp., which has a rigid cell wall. Fry can directly utilize Tetraselmis sp. and Isochrysis galbana, although both are nutritionally inadequate for growth and survival. The nutritional value of rotifers to milkfish larvae varies, depending upon the type of algae that the rotifers are fed. Preliminary studies suggest that Tetraselmis sp. or Isochrysis sp. are preferable to Chlorella sp. as unialgal sources of food for rotifers; however, better results may be obtained when rotifers are fed a mixture of Chlorella sp., Isochrysis galbana, and Tetraselmis sp.

In milkfish larvae, Tetraselmis-fed rotifers produced faster growth and better hepatocyte ultrastructure than Chlorella-fed rotifers. Hence, the dietary value of yeast-fed rotifers may be improved by enrichment culture with Tetraselmis or Isochrysis rather than Chlorella. The nutritive value of an algal feed, however, may be greatly affected by conditions under which it is grown.

The use of copepods or artificial diets for larval rearing of milkfish has not been studied extensively. Inadequate knowledge of larval nutritional requirements has impeded progress in this area. The composition of milkfish egg yolk may be used as a base for feed formulation. Possible effects of maternal spawner diet or of conditions of broodstock keeping on egg yolk composition should be considered.

Abrupt weaning of 2-week-old hatchery-bred milkfish larvae from live foods (rotifers) to artificial diets has been achieved with survival rates comparable to that obtained in larvae fed Artemia sp. nauplii. Application of artificial feeds at even earlier stages appears possible.

Histological methods for nutritional assay of larvae may provide valuable supplementary information to growth and survival studies. More information is needed on the relationships between short-term dietary effects on internal organ ultrastructure and long-term dietary effects on growth and survival.

Elucidating the relationships between the morphological and functional development of the digestive tract in milkfish larvae will be important in the development of appropriate diets for different stages.

Lacking sufficient data on larval nutritional requirements, extensive culture using fertilizer-induced plankton blooms may be practical.

Vision is the most important sense for milkfish fry in feeding. A diurnal variation in feeding pattern was observed in laboratory-reared milkfish larvae under natural light conditions. Optimum light regimes and intensities for rearing milkfish larvae have not been determined.

Optimal concentrations of food organisms to be used during rearing of milkfish larvae also require study.

Mechanoreceptors (lateral line, inner ear) and chemoreceptors (olfactory, gustatory) may play a role in larval food selection. The potential for olfactory and gustatory enhancement or acoustic control of feeding is suggested.

Optimal temperature, salinity and water quality requirements for rearing milkfish larvae have not been determined. Possible deleterious effects of high oxygen levels (i.e., "gas bubble disease") should be investigated.

Studies are needed on rearing tank designs incorporating features which meet the behavioral (e.g., rheotaxis, phototaxis, response to moving particles, swimming speed, etc.) requirements of milkfish larvae with respect to current velocities, manner of food particle presentation and distribution.

Available studies on larval rearing of milkfish have of necessity been empirical in nature. It is difficult to draw any firm conclusions regarding optimal rearing requirements. Although successful rearing of milkfish larvae through metamorphosis with occasionally high survival has demonstrated the feasibility of mass-rearing of this species, more definitive experimental evidence is required to establish the biological data upon which a standardized and reliably transferrable mass-rearing system can be based.

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REFERENCES

Acosta, B.O. and J.V. Juario. 1983. Biological evaluation of Brachionus plicatilis fed Chlorella sp., Isochrysis galbana and Tetraselmis sp. and their combinations as feed for milkfish (Chanos chanos Forsskal) fry. Poster paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines. 4-8 Oct., 1983.

Appelbaum, S. 1985. Rearing of the Dover sole, Solea solea (L.) through its larval stages using artificial diets. Aquaculture 49: 209-221.

Atema, J., 1980. Chemical senses, chemical signals, and feeding behavior in fishes. In: J.E. Bardach, J.J. Magnuson, R.C. May and J.M. Reinhart (Eds.) Fish Behavior and its Use in the Capture and Culture of Fishes. ICLARM, Manila, Philippines. pp. 57-101.

Banno, J.E. 1983. The food and feeding habit of the milkfish fry Chanos chanos (Forsskal) collected from two habitats along the coast of Hamtik, Antique. Paper presented during the Second Internat. Milkfish Aqua. Conf., Iloilo, Philippines, 4-8 Oct., 1983.

Blaxter, J.H.S. 1969. Development of eggs and larvae. In: W.S. Hoar and D.J. Randall (Eds.) Fish Physiology, Vol. 3. Academic Press, New York, N.Y. pp. 177-252.

Blaxter, J.H.S. 1980. Vision and the feeding of fishes. In: J.E. Bardach, J.J. Magnuson, R.C. May and J.M. Reinhart (Eds.) Fish Behavior and its Use in the Capture and Culture of Fishes. ICLARM, Manila, Philippines. pp. 32-56.

Buri, P. 1980. Ecology of the feeding of milkfish fry and juveniles, Chanos chanos (Forsskal) in the Philippines. Mem. Kagoshima Univ. Res. Center S. Pac. 1: 25-42.

Camacho, A.S. 1979. Nutrition of milkfish. Paper presented at the Technical Consultation on Available Aquaculture Technology in the Philippines. Feb. 8-11, 1979. SEAFDEC-PCARR.

Carreon, J.A., L.V. Laureta, F.A. Estocapio and T.U. Abalos. 1984. Milkfish seedling survival in raceways of freshwater recirculating systems. Aquaculture 36: 257-272.

Chaudhuri, H. J.V. Juario, J.H. Primavera, R. Samson and R. Mateo. 1978. Observations on artificial fertilization of eggs and the embryonic and larval development of milkfish, Chanos chanos (Forsskal). Aquaculture 13: 95-113.

Delsman, H.C. 1926. Fish eggs and larvae from the Java Sea. Treubia 8(3-4): 389-412.

Delsman, H.C. 1929. Fish eggs and larvae from the Java Sea. Treubia 11(2): 275-286.

Duenas, C.E. and P.S. Young. 1984. Salinity tolerance and resistance of milkfish larvae. Paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines. 4-8 Oct., 1983.

Duray, M. and T. Bagarinao. 1984. Weaning of hatchery-bred milkfish larvae from live food to artificial diets. Aquaculture 41: 325-332.

Ferraris, R.P., J.D. Tan and M.C. de la Cruz. 1983. The developmental morphology of the digestive tract in the milkfish Chanos chanos Forsskal. Paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines. 4-8 Oct., 1983.

Fujiya, M., S. Sakaguchi and O. Fukuhara. 1980. Training of fishes applied to ranching of red sea bream in Japan. In: J.E. Bardach, J.J. Magnuson, R.C. May and J.M. Reinhart (Eds.) Fish Behavior and its Use in the Capture and Culture of Fishes. ICLARM, Manila, Philippines. pp. 200-209.

Fukusho, K. 1980. Mass production of a copepod, Tigriopus japonicus, in combination culture with a rotifer Brachionus plicatilis, fed w-yeast as a food source. Bull. Jpn. Soc. Sci. Fish. 46(5): 625-629.

Fukusho, K. 1983. Present status and problems in culture of the rotifer Brachionus plicatilis for fry production of marine fishes in Japan. Symposium Internacional De Acuacultura Coquimbo, Chile, September 1983. pp. 361-374.

Fukusho, K. T. Arakawa and T. Watanabe. 1980. Food value of a copepod, Tigriopus japonicus, cultured with w-yeast for larvae and juveniles of mud dab Limanda yokohamae. Bull. Jpn. Soc. Sci. Fish. 46(4): 499-503.

Hara, S., E.M. Avila, T.U. Bagarinao and M.M. Parazao. 1983. Diurnal feeding patterns of milkfish (Chanos chanos) larvae under laboratory conditions. Poster paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines. 4-8 Oct., 1983.

Holliday, F.G.T. 1969. The effects of salinity on the eggs and larvae of teleosts. In: W.S. Hoar and D.J. Randall (Eds.) Fish Physiology, Vol. 1. Academic Press, New York, N.Y. pp. 293-311.

Houde, E.D. 1972. Some recent advances and unsolved problems in the culture of marine fish larvae. Proceedings of the Third Annual Workshop of the World Mariculture Society, St. Petersburg, Florida.

Howell, B.R. 1979. Experiments on the rearing of larval turbot, Scophthalmus maximus L. Aquaculture 18: 215-225.

Hunter, J.R. 1980. The feeding behavior and ecology of marine fish larvae. In: J.E. Bardach, J.J. Magnuson, R.C. May and J.M. Reinhart (Eds.) Fish Behavior and its Use in the Capture and Culture of Fishes. ICLARM, Manila, Philippines. pp. 287-330.

Iwai, T. 1980. Sensory anatomy and feeding of fish larvae. In: J.E. Bardach, J.J. Magnuson, R.C. May and J.M. Reinhart (Eds.) Fish Behavior and its Use in the Capture and Culture of Fishes. ICLARM, Manila, Philippines. pp. 124-145.

Juario, J.V. and M.N. Duray. 1983. A guide to induced spawning and larval rearing of milkfish Chanos chanos (Forsskal). SEAFDEC Technical Rpt. No. 10 (2nd Ed.), September, 1983. 22 p.

Juario, J.V. and M. Natividad. 1980. The induced spawning of captive milkfish. Asian Aquaculture 3: 3-4.

Juario, J.V., M.N. Duray, V.M. Duray, J.F. Nacario and J.M.E. Almendras. 1984. Induced breeding and larval rearing experiments with milkfish Chanos chanos (Forsskal) in the Philippines. Aquaculture 36: 61-70.

Juario, J.V. and V. Storch. 1984. Biological evaluation of phytoplankton (Chlorella sp., Tetraselmis sp. and Isochrysis galbana) as food for milkfish (Chanos chanos) fry. Aquaculture 40(3): 193-198.

Kafuku, T. and Y. Kuwatani. 1976. Physiological functions of the epibranchial organ of milkfish from the point of its ontogeny. Proceedings of the International Milkfish Workshop Conference. Iloilo, Philippines. pp. 47-49.

Kawamura, G. 1984. The sense organs and behavior of milkfish fry in relation to collection techniques. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc. Metro Manila, Philippines. pp. 69-84.

Kawamura, G. and S. Hara. 1980. On the visual feeding of milkfish larvae and juveniles in captivity. Bull. Jpn. Soc. Sci. Fish. 46: 1297-1300.

Kawamura, G. and W. Nishimura. 1980. S-potential from the retina of milkfish Chanos chanos (Forsskal). Bull. Jpn. Soc. Sci. Fish. 46: 1421.

Kitajima, C., S. Fujita, F. Oowa, Y. Yone ad T. Watanabe. 1979. Improvement of the dietary value for red sea bream larvae of rotifers, Brachionus plicatilis, cultured with baker's yeast, Saccharomyces cerevisiae. Bull. Jpn. Soc. Sci. Fish. 45: 469-471.

Kumagai, S. 1984. The ecological aspects of milkfish fry occurrence, particularly in the Philippines. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc. Metro Manila, Philippines. pp. 53-68.

Kuo, C-M. 1982. Progress on artificial propagation of milkfish. ICLARM Newsletter 5(1): 8-10.

Kuo, C-M. 1984. Natural spawning of captive milkfish in Taiwan. ICLARM Newsletter 7(4): 18-19.

Kuo, C-M., Z.H. Shehadeh and K.K. Milisen. 1973. A preliminary report on the development, growth and survival of laboratory reared larvae of the grey mullet, Mugil cephalus L. J. Fish Biol. 5: 459-470.

Kuronuma, K. and K. Fukusho. 1984. Rearing of marine fish larvae in Japan. International Development Research Centre. Ottawa, Canada. 109 pp.

Kuwatani, Y. and T. Kafuku. 1978. Morphology and function of epibranchial organ studied and inferred on milkfish. Bull. Freshwater Fish. Res. Lab., Tokyo. 28: 221-236.

Lam, T.J., J.V. Juario and J. Banno. 1985. Effect of thyroxine on growth and development in post-yolk-sac larvae of milkfish, Chanos chanos. Aquaculture 46: 179-184.

Liao, I.C. 1975. Experiments on induced breeding of the grey mullet in Taiwan from 1963 to 1973. Aquaculture 6: 31-58.

Liao, I.C., J.V. Juario, S. Kumagai, H. Nakajima, M. Natividad and P. Buri. 1979. On the induced spawning and larval rearing of milkfish, Chanos chanos (Forsskal). Aquaculture 18: 75-93.

Lio-Po, G., R.O. Duremdez and A.R, Castillo, Jr. 1984. An investigation of gas bubble disease occurrence among Chanos chanos fry. Paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines. 4-8 Oct., 1983.

Lim, C., S. Sukhawongs and F.P. Pascual. 1979. A preliminary study on protein requirements of Chanos chanos (Forsskal) fry in a controlled environment. Aquaculture 17: 195-210.

Lin, L.-T. 1984. Studies on the induced breeding of milkfish Chanos chanos Forsskal) reared in ponds. China Fisheries 378: 3-29.

Lin, L.-T. 1985. My experience in artificial propagation of milkfish - studies on natural spawning of pond-reared broodstock. In: C.S. Lee and I.C. Liao (Eds.) Reproduction and Culture of Milkfish. Oceanic Institute, Hawaii, U.S.A. and Tungkang Marine Laboratory, Tungkang, Pingtung, Taiwan. pp. 185-203.

May, R.C. 1970. Feeding larval marine fishes in the laboratory: a review. Calif. Mar. Res. Comm., CalCOFI Rep. 14: 76-83.

Morris, R.W. 1955. Some considerations regarding the nutrition of marine fish larvae. J. Cons. Int. Explor. Mer. 19: 255-264.

Nash, C.E. and Z.H. Shehadeh (Eds.) 1980. Review of breeding and propagation techniques for grey mullet, Mugil cephalus L. ICLARM Studies and Reviews 3. ICLARM, Manila, Philippines. 87 pp.

Pantastico, J.B., J.P. Baldia and D.M.T. Reyes. 1983. Feed preference of milkfish (Chanos chanos Forsskal) fry given different algal species as natural feed. Paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines. 4-8 Oct., 1983.

Santiago, C.B., M. Banes-Aldaba and E.T. Songalia. 1983. Effect of artificial diets on growth and survival of milkfish fry in fresh water. Aquaculture 34(3/4): 247-252.

Schuster, W.H. 1960. Synopsis of biological data on milkfish Chanos chanos (Forsskal). 1775. FAO Fish. Biol. Synop. No. 4, Rome FB/60/S4. 6 pp.

Segner, H., B. Orejana-Acosta and J.V. Juario. 1984. The effect of Brachionus plicatilis grown on three different species of phytoplankton on the ultrastructure of the hepatocytes of Chanos chanos (Forsskal) fry. Aquaculture 42: 109-115.

Spectorova, L.V., T.M. Aronovich, S.I. Doroshev and V.P. Popova. 1974. Artificial rearing of the Black Sea turbot larvae (Scophthalmus maeoticus). Aquaculture 4: 329-340.

Storch, V. and J.V. Juario. 1983. The effect of starvation and subsequent feeding on the hepatocytes of Chanos chanos (Forsskal) fingerlings and fry. J. Fish. Biol. 23: 95-103.

Storch, V., W. Stahlin and J.V. Juario. 1983. Effects of different diets on the ultrastructure of hepatocytes of Chanos chanos fry (Chanidae; Teleostei): an electron microscopic and morphometric analysis. Mar. Biol. 74: 101-104.

Storch, V., J.V. Juario and F.P. Pascual. 1984. Early effects of nutritional stress on the liver of milkfish, Chanos chanos (Forsskal), and on the hepatopancreas of the tiger prawn, Penaeus monodon (Fabricius). Aquaculture 36: 229-236.

Tampi, P.R.S. 1958. On the food of Chanos chanos (Forsskal). Indian J. Fish. 5: 107-117.

Teshima, S-I., A. Kanazawa and G. Kawamura. 1984. Effects of several factors on growth of milkfish (Chanos chanos Forsskal) fingerlings reared with artificial diets in aquaria. Aquaculture 37(1): 39-50.

Thorpe, J.E. and J.W.J. Wankowski. 1979. Feed presentation and food particle size for juvenile Atlantic salmon, Salmo salar L. In: J.E. Halver and K. Tiews (Eds.) Finfish Nutrition and Fishfeed Technology. Springer, Berlin. pp. 501-513.

Van Limborgh, C.L. 1979. Industrial production of ready to use feeds for mass rearing of fish larvae. In: J.E. Halver and K. Tiews (Eds.) Finfish Nutrition and Fishfeed Technology. Springer, Berlin. pp. 3-11.

Vanstone, W.E., L.B. Tiro, Jr., A.C. Villaluz, D.C. Ramsingh, S. Kumagai, P.J. Duldcco, M.M. Barnes and C.E. Duenas. 1977. Breeding and larval rearing of the milkfish Chanos chanos (Pisces, Chanidae). SEAFDEC Technical Rpt. No 3: 3-17.

Vicencio, Z.T. 1977. Studies on the food habits of milkfish, Chanos chanos (Forsskal). Fish. Res. J. Phil. 2: 3-18.

Villaluz, A.C. and A. Unggui. 1983. Effects of temperature on behavior, growth, development and survival in young milkfish, Chanos chanos (Forsskal). Aquaculture 35: 321-330.

Villaluz, A.C., W.R. Villaver and R.J. Salde. 1983. Milkfish fry and fingerling industry of the Philippines: methods and practices. SEAFDEC Technical Rpt. No. 9 (2nd Ed.). 81 pp.

Villegas, C.T., O.M. Millamena and F. Escritor. 1983. Growth, survival and fatty acid composition of Chanos chanos fry fed Brachionus plicatilis reared on three selected algal diets. Paper presented during the Second International Milkfish Aquaculture Conference, Iloilo, Philippines. 4-8 Oct., 1983.

Watanabe, T., C. Kitajima and S. Fujita. 1983 Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 34: 115-143.

6. FRY AND FINGERLING COLLECTION AND HANDLING

by

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6-1. INTRODUCTION

The natural fry fishery is one of the foundations of milkfish aquaculture. Information is needed on the status of milkfish fry fisheries and the technological efficiency of the various phases of activity, from catching to stocking. This paper reviews the methods and practices of collection, storage, transport and acclimation of milkfish fry and fingerlings in various countries, and discusses the probable factors affecting catch and survival.

6-2. GENERAL CONSIDERATIONS

6-2.1. OCCURRENCE OF FRY

Spawning of milkfish occurs in the sea not far from shore. Initially, the eggs and resultant larvae are dispersed over the coastal and nearshore oceanic waters. The pelagic larvae ultimately are concentrated and transported by eddies in the general coastal areas where they originated (Johannes, 1980; Kumagai, 1981, 1984). After 2 to 3 weeks, fry (12-15 mm TL) migrate to the shore and enter coastal wetlands. They settle in these areas for about one month until they grow to be juveniles of about 50 mm TL (Villaluz et al., 1982). This particular migration pattern is exploited by fishermen to catch milkfish fry and fingerlings in great numbers along the shore and coastal wetlands.

The milkfish fry season occurs at different times of the year in various sections of the species' geographic range. The fry season is longer near the equator and becomes progressively shorter at higher latitudes. In regions affected by monsoon or trade winds, the peak fry season typically coincides with one or both of the twice-yearly wind shifts. These seasonal peaks are more or less predictable, but fry abundance may vary from year to year. The milkfish fry seasons in several countries are shown in Table 1.

Most milkfish fry are caught one to two days before, and up to three days after the new moon and full moon periods (Kuronuma and Yamashita, 1962; Kumagai et al., 1976; Kumagai, 1981; Noor-Hamid et al., 1977). This periodic increase in fry catch is caused by more intense spawning of milkfish during the quarter moon periods. The fry catch is also observed to increase when wind direction is toward the shore. Schmittou (1977) suggested that this could be partially caused by the increase in volume of surface water reaching the shore.

Table 1. Peak seasons and estimated annual catch of milkfish fry in several countries.

Location	Occurrence	Peak Season Major	Minor	Annual Catch	Source
Philippines	Jan-Dec		-	1.15-1.35 billion	Smith, 1981; Villaluz et al., 1983
North	Mar-Aug	May-Jul			
Central	Mar-Jan	Apr-Jun	Nov		
South	Jan-Dec	Mar-May			
Indonesia	Jan-Dec			740 million	Noor-Hamid et al., 1977; Chong et al., 1984
North	Apr-Nov	Apr-May			
Central	Mar-Dec	Aug-Oct	Apr-Jun		
South	Dec-Feb	-	-		
Taiwan	Apr-Sep	May	Aug	34-235 million	Lin, 1969; Lee, 1984
India	Mar-Nov	May	Oct-Nov		Schuster, 1960
Sri Lanka	Mar-Nov	Apr-May	Oct-Nov	400 million*	Ramanathan, 1969
Thailand	Mar-Dec	Apr-May	Sep		Thiemmedh, 1955
Vietnam	Apr-Oct				Kuronuma & Yamashita, 1962
Japan	Jun-Sep	Jul-Aug			Senta et al., 1980
Fiji	Feb-Mar				Schuster, 1960
Kiribati	Jan-Dec	Apr	Sep		Hoogester, 1982
Panama	Mar-Jul				Villaluz, 1983

* potential fry catch

Milkfish fry arrive in shore waters in patches and generally utilize longshore and tidal currents as a passive means of transport (Buri and Kawamura, 1983), but they may actively enter coastal wetlands even during receding tides (Villaluz et al., 1983). Catch is better during flood tides because the fry remain far from shore after high tides. This makes the fry inaccessible to most types of gear during falling tides.

6-2.2. CAPTURE OF FRY

Kawamura et al. (1980) concluded from behavioral observations that vision is important in net avoidance by fry. The fry are not caught by filtering but by driving. Field tests of catching gear with highly visible (black colored) nets, having larger mesh and wing dimensions resulted in a drastically reduced catch, however. These poor catches were probably caused by cancellation of the driving effect of the nets by the relatively turbid water of the shore during the fry season.

Current developments and modifications of milkfish fry gear are directed to better performance in deeper areas of the fry grounds. Encina and Gatus (1977) reported that milkfish fry congregate close to fish shelters located offshore and can be caught in sizable quantities in deeper coralline areas. Where the water is clear, fry collection gear that effectively utilizes both the driving effects of the net and the optomotor responses of the fish should be used (Kawamura et al., 1980; Kawamura, 1984).

Milkfish fry stay mainly at or near the water surface even when scared (Villaluz et al., 1983; Buri and Kawamura, 1983). The present depths of all types of catching gear, therefore, do not have to be increased to catch more fry.

6-3. COLLECTION GEAR AND METHODS

Design, construction and area of operation of milkfish catching gear are primarily dictated by the topography of the fry ground, wind direction, current patterns and tidal fluctuations. The behavior of the fish determines the collection methods to be employed in specific areas. Modifications of the gear and methods of collection are directed to suit the convenience of fry collectors as well as by the availability and cost of materials.

Traditional and current fishing gear and methods of catching milkfish fry and fingerlings in the Philippines (Villaluz, 1953; Bunag, 1957; Encina and Gatus, 1977; Kumagai et al., 1980; Villaluz et al., 1983), Indonesia (Schuster, 1952; Noor-Hamid and Mardjono, 1976; Noor-Hamid et al., 1977; Chong et al., 1984), Taiwan (Lin, 1969; Lee, 1984), Sri Lanka (Ramathan, 1969; Villaluz et al., 1982; Thayaparan and Chakrabarty, 1984), Panama (Villaluz, 1983), and Thailand (Thiemmedh, 1955) are presented below.

6-3.1. FRY BARRIERS OR FENCES (Fig. 1)

These are devices to which fry are attracted or on which they are concentrated by favorable winds and tidal currents. The gear may consist of fine mesh (0.3-1.6 mm) nylon netting, split bamboo netting, coconut palm or any combination thereof, tied to bamboo or mangrove stakes. Fry barriers or fences usually are set perpendicular to the shore, extending 10-50 m into the water. Shallow intertidal areas along a narrow pass, or tidal flats with muddy coralline substrate are suitable locations for these devices. The gear is also set halfway across tidal creeks. Actual catching of fry is accomplished by skimming nets or double stick nets. This method is used in the Philippines and Indonesia.

In Indonesia, gear consisting of dried grass or banana leaves woven into a rope is used both as a fry barrier and also to drive milkfish fry into shallow water. The 20 m rope is laid in a wide circle and the diameter of the enclosed area is gradually reduced by pulling one end of the rope while the other end is fastened to a stake. The fry are caught by the skimming net in the small central portion of the closed ring.

6-3.2. FILTER BAG NETS

1. Skimming Net (Fig. 2): The gear has triangular or semicircular frames. The nets may be made of abaca cloth (fiber 0.2-0.4 mm, mesh 0.3-1.3 mm) or fine mesh nylon netting. Skimming nets filter milkfish fry from the water when the operator pushes the net forward. This is usually used in mangrove areas, along fishpond dikes and canals and in tidal flats with a muddy or coralline substrate. Women and children prefer it because it is lightweight and easy to operate. It has been introduced in Sri Lanka and Panama, but is mainly used in the Philippines, Taiwan and Indonesia.

2. Tidal Set Net (Fig. 3): The gear consists of fixed wings made of matted bamboo or fine mesh nylon netting and a catching chamber made of either abaca cloth or fine mesh nylon netting. Tidal set nets are constructed across the mouth of a river or creek and occasionally along the shore facing favorable wind and tidal currents. A series of two to four tidal set nets may be placed across the mouth of a relatively large river. The gear is generally operated by two to five men during flood tide. In some places, the catching operation is continued even during receding tides when milkfish fry swim against the outgoing current. Although this gear has been introduced in Indonesia and Thailand, its use for commercial fry collection is limited to the Philippines.

3. Floating Tidal Set Net (Fig. 4): The gear consists of V-shaped floating wings made of coconut leaves tied to bamboo poles and connected to a bamboo frame. The sides are made of fine mesh nylon netting and the catching chamber is made of abaca cloth. The offshore side of the wings is longer (20-30 m) than the nearshore side (6-8 m). The opening between the two tips of the wing is from 20-30 m.

This is set against longshore currents and is suited particularly to coastal promontories with relatively shallow coralline platforms. In certain areas, several units may be set about 10-15 m apart, one in front of the other. The gear is usually operated by one man during flood tide, but operation is continued even during the receding tide when wind or current direction is toward the gear. This method is used only in the Philippines.

4. Push Net (Fig. 5): This gear consists of a V-shaped bamboo frame provided with a detachable fine mesh nylon netting and a bagnet located at the narrow end. Abaca cloth is usually sewn over the nylon netting at the end portion of the bagnet to prevent the fry from sticking during the process of concentrating and scooping. The wings and bagnet are weighed down with lead sinkers. The top portions are tied to the frame above the water line, while the lower parts are 5-15 cm below the water surface. The gear is usually operated along the shore or river banks by two people--one pushing the gear and scooping the fry from the bagnet and the other shuttling and sorting the fry. In some areas, the push net has bigger dimensions and is provided with a platform for basins and pails. In this case, one person pushes the gear while the other pulls it with a rope from the shore. The push net is also used in a stationary position along the shore or river mouth and is operated like the tidal set net. On stormy days, push nets are set against the surf. The push net has been motorized in some areas in Taiwan and the Philippines. In the latter, it is modified by replacing the wings with bigger mesh size nylon netting (4 mm) to reduce water resistance, and then attached to one side of a pumpboat with a 10-16 hp engine and used in the deeper portions of fry grounds. The push net has been demonstrated in Indonesia, but there is no report indicating whether it has been adopted by local fry collectors.

5. Push net with Bamboo Raft (Fig. 6): This gear is similar to the push net described above except it is provided with a bamboo raft. The wings are made of matted bamboo attached to whole bamboo logs which serve as floats. The bagnet is made of abaca cloth. This gear is usually operated in fry grounds with extensive shallow intertidal waters up to 5 m deep. Two people are required for operation. One person pushes the gear with a bamboo pole while the other scoops and sorts the fry. This method is usually undertaken at night until early morning (2100-0400 h), with a lamp being used to facilitate catching and sorting of fry. At times, this gear is set along the river bank and is operated like the tidal set net. This gear and method have been reported only in Panay Island, Philippines, however, a motorized push net with bamboo raft is common in Taiwan.

6. Tow Net with Bamboo Floats (Fig. 7): This gear is also similar to the push net, but, instead of a rigid bamboo frame, two bamboo poles are used as floats. The wings and bagnet are made of fine mesh nylon netting; abaca cloth is sewn over the scooping end of the bagnet. The mid-front section of the bagnet is provided with a series of lead sinkers. Each wing-end is attached to a bamboo pole to facilitate towing and to keep the wings in an upright position while in operation. The gear is towed along the shore by two people, one at the free end of each wing. When one of the operators takes the fry to shore, the other continues towing by holding the free ends of both wings. This gear is also used in rivers or creeks, either mobile or operated like a tidal set net. This method has been used only in southern Luzon, Philippines.

7. Tow Net (Fig. 8): This gear consists of wings made of fine mesh netting and a cylindrical bag made of sail cloth. A float is attached at the upper mid-portion of the bagnet's opening, while the lead sinkers are placed at the

lower edge. Each wing-end has a bamboo pole with a 1.0 m nylon rope tied to the lower portion of the pole. This keeps the wings in vertical position when the gear is subjected to undercurrent. The tow net is used in areas with steep shore profiles, high waves and stormy winds. Two people operate it, each pulling on the bamboo pole at the end of one wing. The filtered fry are concentrated at the cod end; the strings at the cod end are untied and the fry are poured into a plastic bag. The tow net can also be operated like a tidal set net in places where a sandbar has formed parallel to the shore or at the mouth of a river or creek. This gear and method have been reported only in the Philippines, but have recently been introduced in Sri Lanka and Panama.

6-3.3. SEINE NETS

1. Double Stick Net (Figs. 9 and 10): This gear usually consists of a rectangular abaca or organdy cloth section (1.0-1.5 m wide and 4.0-6.0 m long) suspended between side bamboo poles. This is towed along the shore or at the river mouth by two people. Various modifications have been made to the original design because of the cost of materials, prevailing local conditions, and mode of operation. Modifications include using fine mesh nylon netting at the wings while retaining abaca cloth in the center portion where the fry are concentrated and scooped. In fry grounds with moderately steep shore profiles, the bamboo poles at one or both ends are replaced with rope loops, allowing the collector to swim with the net. A smaller version of the double stick net is used by children, mostly in shallow rivers or creeks. This type of gear is used in all countries where milkfish fry abound.

In Sri Lanka, milkfish fry and fingerlings in pools at the higher marsh level of coastal wetlands are also collected with the double stick net. With this method of capture, the area is first searched for schools of milkfish. The collector then scares the fish with shadows cast by waving hands. This drives the school to shallower areas and the gear is towed toward the school when the fish have formed a "ball". The fish are concentrated at the center of the gear and transferred to a container with a scooping bowl.

2. Fry Seine Net (Figs. 11 and 12): This gear consists of equal towing lines at both ends of coarse mesh (1.6 mm) nylon netting wings and a concentrating section made of fine mesh nylon netting with abaca cloth sewn over the center. The gear is provided with a series of floats at the top and lead sinkers at the bottom. Two people operate this net, one staying on the shore holding one end of the towline while the other casts the gear 50-100 m from the shore with the use of a small boat. The fry are concentrated and scooped at the shore. The fry seine net is usually operated near river mouths during low tides and neap tides and set consecutively or repeatedly until the catch becomes minimal. This gear has been modified with a bagnet at the center similar to the tow net with bamboo floats. The wings are longer and made entirely of fine mesh nylon netting. Four people are required to operate the gear, which is used only in Antique Province, Philippines.

6-4. STORAGE OF FRY

6-4.1. GENERAL PRACTICE

The general practice for storing milkfish fry is similar in Indonesia (Noor-Hamid and Mardjono, 1976; Noor-Hamid et al., 1977), Taiwan (Lin, 1969), and the Philippines (Villaluz et al., 1983) and is described below.

The whole catch, which may include other fish fry and crustacean species, is brought to shore after capture. It is then transferred to an earthen jar, a white basin, a water-tight bamboo basket, or a palm basket. A small cup, bowl or shell is used for counting and sorting. The counted fry are placed in another container while debris, dead fry and

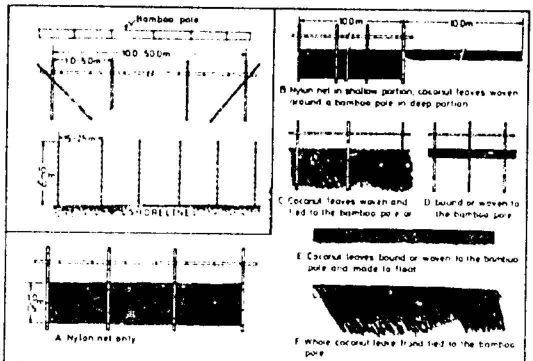

Fig 1 Milkfish fry barrier or fence, its structure and dimensions(inset), and kinds of materials used (A-F)

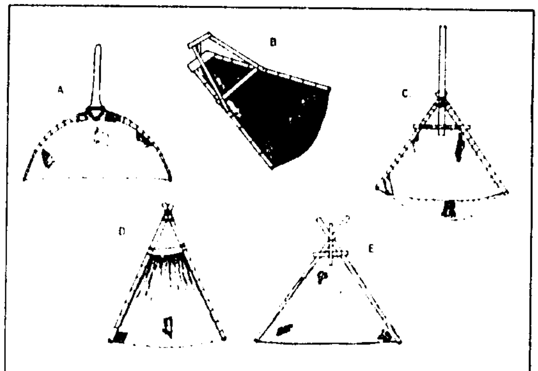

Fig 2 Different modifications of skimming net

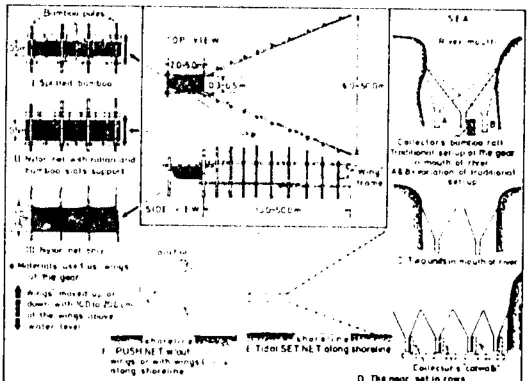

Fig 3 Tidal set net, its structure and dimensions(inset), wing materials (I-III), and variations in setting up(A-F)

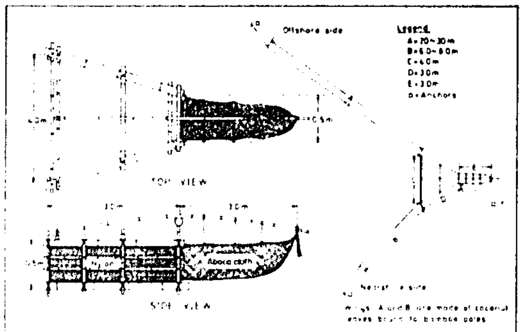

Fig 4 Floating tidal set net, its structure, dimensions and how it is set up

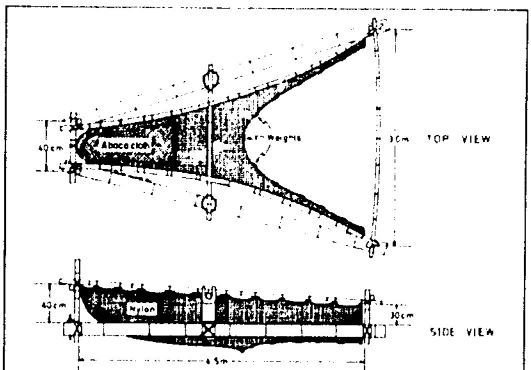

Fig 5 Push net, basic type, its structure and dimensions

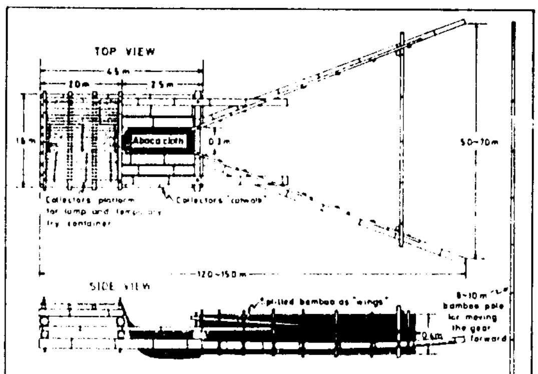

Fig 6 Push net with bamboo raft, it structure and dimensions

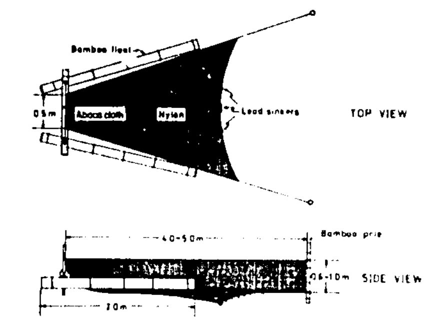

Fig 7 Tow net with bamboo floats, its structure and dimensions

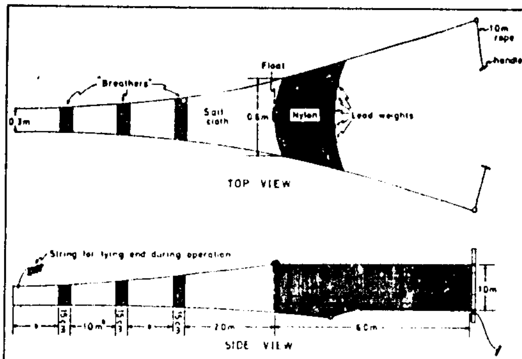

Fig 8 Tow net, its structure and dimensions

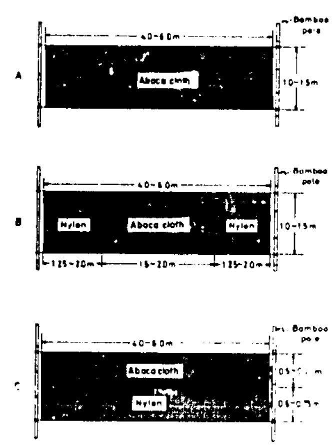

Fig 9 Modifications of double stick net

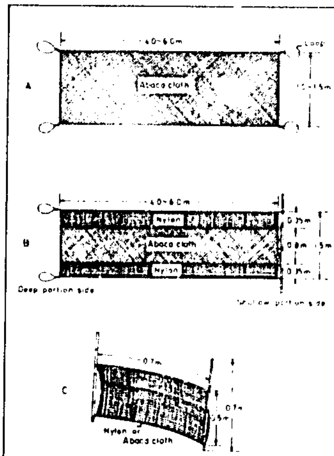

Fig 10 Modifications of double stick net

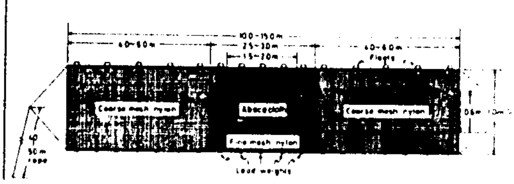

Fig 11 Fry seine net, structure, dimensions and materials used in the construction

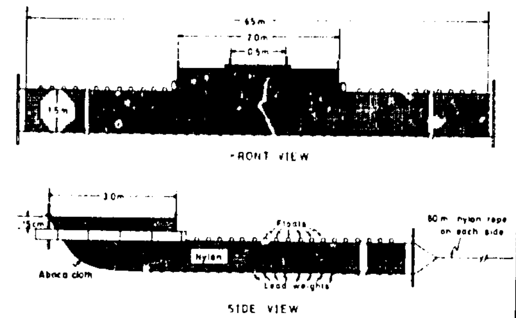

Fig 12 Modified fry seine net, its structure and dimensions

unwanted species are discarded. In the Philippines, a cylindrical device made of nylon netting is utilized to sort fry if undesirable organisms are numerous.

Fry of tarpon (Megalops cyprinoides) and ten-pounder (Elops machnata) look very similar to milkfish larvae. They are voracious predators of milkfish fry and fingerlings in the nursery pond. Both can be distinguished from the milkfish by their longer and wider bodies, swimming movements and light amber color.

An actual head count is done when fry are few, but when the catch runs into several thousands, counting is done with the aid of pebbles, shells or any suitable material. One small pebble or shell represents 100 fry, while a bigger one represents 1,000 fry. Another method of counting is done by visual estimation; the density of fry in one container is compared with density of fry of a known number in another container.

The fry are not fed during overnight storage after being caught. The storage water generally is diluted with freshwater. In the Philippines, the ratio of dilution is three parts seawater to one part freshwater. The fry, now in oxygenated plastic bags, earthen jars, or bamboo and palm baskets (10-301 cap.), pass to a dealer and a longer-term storage follows. The water in the storage container (20-30 liter capacity) is diluted again at ratios of one part seawater to one part freshwater to approximate 20-25 ppt in the Philippines, and one part seawater to four parts freshwater in Indonesia. Another method of obtaining the desired water medium for fry storage in Indonesia is by mixing salt with freshwater to obtain a salinity of 10 ppt. The fry containers are then covered to minimize fry activity and to prevent dirt from entering the containers.

Food, such as the yolk of hard boiled eggs, whole poached eggs, pulverized rice, or dried wheat flour, is given to the fry either daily or every other day. The storage containers are inspected every morning and afternoon; the excess food, feces, dead fry and debris are removed. Before cleaning, the water in the container is swirled by hand to concentrate the unwanted materials at the center. A bowl or shell is then used to scoop out these potentially toxic wastes. Every day or every other day, storage water is either completely or partially replaced with new water premixed at the desired salinity level.

Fry stored for more than 15 days are usually weak. Very low survival is obtained when these are stocked in the nursery pond. The condition of the fry may be determined by the following procedures:

1. Observe the fry closely. Strong and healthy fry move continuously in the same direction along the wall of the container. If the fry display this behavior only occasionally or swimming is sluggish, the fry are weak.

2. Swirl the water. Healthy fry will swim against the current.

3. Tap the container or move a hand over it. Fry that react with a quick diving movement are in good condition.

Some dealers in the Philippines and Indonesia keep fry alive and in reasonable condition for one to three months by storing them in earthen jars (20-30 liter capacity) and reducing the stocking density to a few hundred per container.

Mortality of fry while in storage may be caused by one or more of the following: physical injuries during catching and handling; overcrowding; water fouling caused by decomposition of food, feces, dead fry, debris, and high bacterial load of storage water; temperature and salinity shock; starvation; storage of longer than 15 days in relatively crowded conditions; and predation by other fish species.

Milkfish fry storage practices in the Philippines, Indonesia and Taiwan are summarized in Table 2.

Table 2. Milkfish fry storage in the Philippines, Indonesia and Taiwan.

Conditions	Fry Storage Practices Philippines	Indonesia	Taiwan
Container	a. plastic basin b. earthen jars	a. earthen jars b. bamboo basket	a. plastic basin b. bamboo baskets
Water Volume (liter)	a. 15-23 b. 10-20	a. 10 b. 30	no report
Salinity (ppt)	10-25	10-25	< 20
Feeds and feeding	egg yolk or wheat flour every day or every other day	rice, flour, dried wheat, or egg yolk	no report
Water management	complete change or 1/2 of total volume every day or every other day	complete change	no report
Stocking rate (fry/container)	a. 3,000-8,000 b. 2,000-3,000	a. 1,000 b. 15,000	no report
Stocking density	a. 150-500 b. 100-300	a. 100 b. 500	no report
Days of storage	1-7	10-20	no report
Mortality (%)	2-10	5-10	< 2
Source	Villaluz et al., 1983	Noor-Hamid et al., 1977	Lin, 1969

6-4.2 SURVIVAL DURING STORAGE

Initial exposure of fry to low salinity (20-25 ppt) results in better survival during storage regardless of salinity levels used (Quinitio and Juario, 1980). Tissue fluid osmolality of fry collected from the shore has an osmotic pressure equivalent to a salinity of 13.67 ppt (Almendras, 1982). The usual practice of dilution with fresh water brings the salinity of the medium close to that of the fry's body fluid, thus minimizing osmotic imbalance. It is then apparent that addition of fresh water not only enhances oxygen availability from the medium, but more importantly, alleviates osmotic stress among the fish.

If the fry are starved for 3 days, they become weak, emaciated and transparent. Mortality would begin on the eighth day if the fry are completely deprived of food. More fry can be stored in plastic basins (300-500 fry/liter) than in earthen jars (100-200 fry/liter) because of a greater surface/volume ratio in the former. For long-term storage (>15 days), earthen jars are more appropriate because their cooler and darker environments decrease activity and consequently the fry's metabolism. This, together with a low stocking density (25 fry/liter) and a salinity of 28-30 ppt, reduces stress and enables the fry to conserve energy while in storage. Vigorous and quality fry are attributed to skillful care and handling in Taiwan (Lee, 1984), some parts of Indonesia (Chong et al., 1984), and the Philippines (Villaluz et al., 1983).

6-5. TRANSPORT OF FRY AND FINGERLINGS

6-5.1. PROCEDURES

The procedures for transporting milkfish fry in Indonesia (Schuster, 1952; Noor-Hamid et al., 1977) and the Philippines (Villaluz et al., 1983) follow. The fry are not fed before transport. The storage containers are cleaned and the water is completely replaced. The fish are transferred to smaller basins and their number determined by visual estimation. Freshwater is usually added to reduce the salinity. In the Philippines, the ratio is one to two parts freshwater to two parts storage water, and in Indonesia, nine parts freshwater to one part seawater is common. Excess water is removed with a small bowl, shell or cup held over a scoop net or nylon netting to exclude the fry. The fry are introduced inside double plastic bags. Oxygen is added at a volume of 1/2 to 2/3 of the water in the bags. The bags are then placed inside palm bags or cardboard boxes if they are transported by land, or inside styrofoam boxes or jerricans in the case of air transport. Transport by boat using earthen jars or bamboo baskets as fry containers is the usual practice in Indonesia. Since it takes 3 to 7 days for the fry to reach their destination, water in the container is changed and the fry fed daily.

In Sri Lanka, the fry are placed directly into double cylindrical plastic bags containing one part lagoon water diluted with one to three parts freshwater. Each bag contains about 4-6 liters of water and 8-12 liters of oxygen. The bags are not placed in containers as in other countries; they are simply arranged vertically inside a jeep or van (Villaluz et al., 1982).

As a general rule, the transport route for fry in Taiwan is short and the fishermen take good care of the fry (Lin, 1969; Lee, 1984). Survival during this phase of the operation is 98%.

Methods of milkfish fry transport in several countries are presented in Table 3.

Transport of milkfish fingerlings has been reported only in the Philippines (Villaluz et al., 1983) and Sri Lanka (Villaluz et al., 1982). The procedures for transporting milkfish fingerlings are similar to that for fry and include the use of plastic bags and oxygen. Dilution of transport water with fresh water, however, is not generally practiced in the Philippines. High mortality is encountered when the fingerlings are stocked in freshwater ponds or pens. The recent innovation of transporting the fingerlings in water

Table 3. Methods of milkfish fry transport in the Philippines, Indonesia, Taiwan and Sri Lanka.

	Fry Transport Practices			
Conditions	Philippines	Indonesia	Taiwan	Sri Lanka
Container	a. plastic bag b. earthen jar	a. plastic bag b. bamboo basket c. earthen jar	a. plastic bag b. bamboo basket	plastic bag
Mode of Transport	a. land, b. air	a. air, b. & c. water	land	land
Transport Time (h)	a. 2-14, b. 3-6	a. 12, b. 4-7 days	1-3	5-10
Water Volume (liters)	a. 8-10, b. 3-5	a. 10, b. 20, c. 30	no report	4-6
Salinity (ppt)	12-22	10-15	<20	10-30
Stocking Rate (fry/container)	a. 4,000-6,000 b. 4,000-8,000	a. 10,000-20,000 b. 1,000 c. 15,000-40,000	no report	1,500-2,000
Stocking Density (fry/liter)	a. 400-750 b. 800-2,000	a. 1,000-2,000 b. 500 c. 500-1,300	no report	375-500
Mortality (5)	2-6	a. 5, b. & c. 20	<2	2-20
Sources	Villaluz et al., 1983	Schuster, 1952; Noor-Hamid et al., 1977; Noor-Hamid and Mardjono, 1976	Lin, 1969; Lee, 1984	Villaluz et al., 1982

with a salinity of 2-5 ppt has considerably reduced mortality during transport and after stocking in fresh water.

Another method of fingerling transport in the Philippines is by means of a "live boat". The boat has a flat bottom used as the fingerling compartment and is provided with two to three holes for free entrance of water. A water pump is used to continuously change the water in the compartment. When passing muddy or polluted water, the holes are closed and the pump recirculates the water inside. Upon reaching the destination, the fingerlings are caught with a fine mesh seine and transferred by pails directly to fishponds or pens. Bad weather, use of polluted or muddy water, overstocking, and sick and weak fish are avoided to prevent mass mortality during or shortly after transport.

The methods of transporting milkfish fingerlings in the Philippines and Sri Lanka are summarized in Table 4.

6-5.2. SURVIVAL DURING TRANSPORT

Temperature is the most critical factor in transport of fry and fingerlings because of its effect on metabolic rate. Oxygen consumption of milkfish fry increases by about 5 times when temperature is elevated from 20^{o} to $32^{o}C$ (Millamena and Villaluz, unpub.). If the number and/or size of fish is small, the dissolved oxygen in the water does not become limiting. A temperature increase in a crowded situation, however, may result in severe stress, if not mass mortality. Solubility of oxygen in water also decreases as temperature and salinity rise. This indicates that stocking density may be higher and time of transport longer if water temperature and salinity are lower.

Increased activity of the fish during transport may cause lactic acid to accumulate in the muscle tissue of the fish and lead to severe oxygen debt (Black et al., 1962). Water and mineral balance (Eddy, 1981) and resistance to

Table 4. Methods of milkfish fingerling transport in the Philippines & Sri Lanka.

Conditions		Fingerling Transport Practices: Philippines, Traditional Method	Philippines, New Method	Sri Lanka
Container	a. b.	plastic bag "live boat"	plastic bag	plastic bag
Mode of Transport	a. b.	land water	land/water	land
Transport Time (h)	a. b.	3-6 4-5	3-6	5-10
Water Volume (liter)		10-15	10	4-6
Salinity (ppt)	a. b.	10-35 initial (20-30 ppt) final depends on where fish are stocked (0-30 ppt)	2-5	10-50
Stocking Rate	a.	500-600 (fish 3-4 cm TL), 200-300 (5-10 cm)	1,500 (fish 3-5 cm TL), 700 (6-8 cm)	600-800 (fish 2.5-3.5 cm FL), 200-400 (4.0-8.6 cm)
Stocking Density (fish/liter)	a. b.	33-60 (3-4 cm) 15-30 (5-10 cm) 20-30 (3-5 cm) 10-12 (6-10 cm)	150 (3-5 cm) 70 (6-8 cm)	100-600 (2.5-3.5 cm) 40-80 (4.0-8.6 cm)
Mortality (%)	a. b.	no report 0.05-2	<1	2-100
Source		Villaluz et al., 1983	Villaluz et al., 1983	Villaluz et al., 1982

A. Traditional Method - Fish not acclimated before transport.
B. New Method - Fish acclimated to 5 ppt for 3-4 days before transport.

diseases (Wedemeyer and McLeay, 1981) may also be impaired. These may cause the fungal infestations and mass mortality still occurring a few hours or days after stocking of fry or fingerlings--even in environments with optimal conditions. Physical injuries also cause mortality during and after transport. Milkfish fry and fingerlings tend to concentrate in corners, creating death traps or causing serious injury while the fish are in transit.

6-6. ACCLIMATION AND STOCKING OF FRY AND FINGERLINGS

Upon delivery to the fishpond, the milkfish fry are usually transferred to another container (20-30 liter capacity) for conditioning, final counting and sorting. The water in the conditioning container is diluted about 25% with pond water 4 to 6 hours after arrival. This is repeated every 2 to 4 hours until the salinity more or less equals that of the pond. Most fish farmers stock fry directly from the container to the pond, while others let the container float in the pond for about 10-15 minutes to further reduce temperature differences. The survival rate is 95-100% after one day of stocking (Villaluz et al., 1983). If the fry are stocked directly upon arrival, the transport containers are made to float in the nursery while the transport water is gradually diluted or replaced with pond water. The fish are released into the nursery 15-20 minutes after arrival and survival after stocking is from 20-100% (Schuster, 1952; Villaluz et al., 1982; Villaluz et al., 1983).

Milkfish fingerlings transported inside plastic bags are acclimated to the pond in the same manner as fry. Heavy mortality follows if this method is utilized in stocking fingerlings to fresh water. In this case, the fingerlings are stocked immediately after capture from the nursery into tanks with a salinity of 5 ppt. They are held in this condition for 3-4 days before transport. Stocking to a freshwater pond or pen is done immediately, without acclimation. Survival is 98-100% one week after stocking (Villaluz et al., 1983). If the "live boat" method of transport is used, acclimation to freshwater is done by gradual, continuous replacement of water in the fingerling compartment at 0.15-0.25% per minute. A mortality rate of 20-30% in the first week after stocking, traceable to improper acclimation, has been reported in Laguna de Bay, Philippines (Villaluz et al., 1983).

Young milkfish can tolerate transient exposure to high temperatures up to 42°C (Pannikar et al., 1953), but daily exposure to temperatures that increase from 25°C to 34°C at 1°/h may be lethal (Villaluz and Unggui, 1983). This shows that the duration of exposure to thermal stress may be more critical to survival than the magnitude of temperature change. On the other hand, milkfish fry have a high recovery potential from thermal stress if transferred immediately to the original temperature.

The water and mineral balance of milkfish fingerlings generally stabilizes 60 hours after transfer to different salinities and fry stabilize in 24 hours (Almendras, 1982). This explains, in part, the apparent susceptibility of milkfish fingerlings and the tolerance of fry to subsequent stress if imposed over a short period of time.

6-7. MARKETING AND DISTRIBUTION OF FRY AND FINGERLINGS

The common marketing practice in Indonesia for milkfish fry has been reported by Chong et al. (1984) (see also Chapter 10). Transport of fry is mostly by water and sometimes by air. Land transport is limited to short distances within an island. The main fry grounds are Aceh in Sumatra, Maluku, Bali, Nusa Tenggara and South Sulawesi. At present, only about 740 million fry are collected from throughout the country. For the most part, these fry are shipped to Java.

Lee (1984) reported that in Taiwan, the fry are commonly transported by motorcycle, taxi, truck or train depending on the distance and the quantity purchased. The main milkfish fry grounds of Taiwan are located in the eastern and southern coast and account for 66% and 31% of the total fry catch, respectively. The primary demand for fry comes from the Tainan area, with Tainan City considered the fry trading center of the country. Wide annual fluctuations in fry supply occur: from a low of 33.96 million (1967) to a high of 234.87 million (1970). Since 1970, fry catch has decreased yearly to about 101.42 million in 1982.

In the Philippines, the marketing and distribution of milkfish fry and fingerlings have been described by Smith (1981). Interisland transport of fry is primarily by air, but water transport is also common between nearby islands. Land transportation is usually utilized within an island. The major transport of fingerlings from the nursery to grow-out ponds or pens is by the "live boat" method. Milkfish fry can be collected in almost all coastal waters of the Philippines. Most of the fry (62.3%) comes from the southern island of Mindanao, while the main nursery pond areas are located in Rizal and Bulacan Provinces in Luzon. Yearly milkfish fry catch is estimated to be from 1.15 to 1.35 billion.

6-8. RECOMMENDATIONS

Methods for increasing fry catch without depleting natural stocks might include:

1. Shoals and waters surrounding islets which are breeding grounds for milkfish (Kumagai, 1981) should be declared fish sanctuaries.

2. Coastal wetlands (lagoons, inland bays, mangroves, rivers, creeks and tidal pools) used as nursery and feeding grounds (Villaluz et al., 1982) should be left undisturbed and not converted into fishponds or utilized for other purposes.

3. Catching adult milkfish migrating from lakes (e.g. Naujan and Taal Lakes in the Philippines) should be regulated to assure replenishment of adult stocks into the sea.

4. Improved gear and strategies for fry capture should be developed and tested.

5. The population dynamics of wild milkfish should be studied to determine optimal fishery yields of fry.

6. Better methods and practices for collection, storage, transport and acclimation of milkfish fry and fingerlings should also be developed.

REFERENCES

Almendras, J.M.E. 1982. Changes in the osmotic and ionic content of milkfish fry and fingerlings during transfer to different salinities. M.S. thesis, University of the Philippines.

Black, E.C., A.R. Connor, K.C. Lam and W.G. Chui. 1962. Changes in the glycogen pyruvate lactate in rainbow trout (Salmo gairdneri) during and following muscular activity. J. Fish. Res. Bd. Cdn. la: 409-436.

Bunag, D.M. 1957. "Bangus" fry trawl. Proc. Indo-Pacific Fish. Coun. (11-111): 77-81.

Buri, P. amd G. Kawamura. 1983. The mechanics of mass occurrence and recruitment strategy of milkfish Chanos chanos (Forsskal) fry in the Philippines. Mem. Kagoshima Univ. Res. Center S. Pac. 3: 33-56.

Chong, K., A. Poernomo, F. Kasryno. 1984. Economic and technological aspects of the Indonesian milkfish industry. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing, Inc. Manila, Philippines. pp. 199-213.

Eddy, F.B. 1981. Effect of stress on osmotic and ionic regulation in fish. In: A.D. Perckering (Ed.) Stress and Fish. Academic Press, London. pp. 77-122.

Encina, V.B. and A.R. Gatus. 1977. Preliminary report on milkfish floating trawl experiment in Balayan Bay. Phil. J. Fish 15: 174-216.

Hoogester, J. 1982. Report on milkfish (Chanos chanos) fry survey at Christmas Island -- Kritimati. Report to the Government of Kiribati dated February, 1982. 18 p.

Johannes, R.E. 1980. Using knowledge of the reproductive behavior of reef and lagoon fishes to improve fishing yields. In: J.E. Bardach, J.J. Magnuson, R.C. May, and S.M. Reinhart (Eds.) Fish Behavior and Its Uses in the Capture and Culture of Fishes. ICLARM Conf. Proc. 5. ICLARM, Manila, Philippines. pp. 247-270.

Kawamura, G. 1984. The sense organ and behavior of milkfish fry in relation to collection techniques. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing, Inc. , Manila, Philippines. pp. 69-84.

Kawamura, G., S. Hara and T. Bagarinao. 1980. A fundamental study on the behavior of milkfish fry for improving the efficiency of traditional fry collecting gear in the Philippines. Mem. Kagoshima Univ. Res. Center S. Pac. 1: 65-74.

Kumagai, S. 1981. Ecology of milkfish with emphasis on reproductive periodicity. Terminal Report to SEAFDEC Aquaculture Dept. 1)6 pp.

Kumagai, S. 1984. The ecological aspects of milkfish fry occurrence, particularly in the Philippines. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing, Inc. , Manila, Philippines. pp. 53-68.

Kumagai, S., T. Bagarinao and A. Unggui. 1980. A study on the milkfish fry fishing gears in Panay Island, Philippines. SEAFDEC Tech. Rep. No. 6. 34 pp.

Kumagai, S. A.C. Villaluz, L.B. Tiro, Jr. and W.E. Vanstone. 1976. The occurrence of milkfish (Chanos chanos) fry in Pandan Bay, Antique, May 21-June 25, 1975. Proc. Int. Milkfish Workshop Cong. Tigbauan, Iloilo, Philippines. pp. 50-57.

Kuronuma, K. and M. Yamashita. 1962. Milkfish fry on eastern coast of Vietnam. J. Oceanog. Soc. Japan, 20th Anniv. Vol. pp. 679-686.

Lee, C.S. 1984. The milkfish industry in Taiwan. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing, Inc., Manila, Philippines. pp. 183-198.

Lin, S.Y. 1969. Milkfish farming in Taiwan. Fish Culture Report No. 3., Taiwan Fisheries Res. Inst. p. 63.

Noor-Hamid, S. and M. Mardjono. 1976. An improved method of collecting gear. Bull. Shrimp Culture Res. Center 2.

Noor-Hamid, S., B. Martosudarmo and M. Mardjono. 1977. Report on the status and occurrence of milkfish fry in Indonesia. Bull. Brackishwater Aqua. Dev. Center 3: 258-267.

Quinitio, G.F. and J.V. Juario. 1980. Effects of various salinity levels and stock manipulation methods on the survival of milkfish fry (Chanos chanos) during storage. Fish. Res. J. Philippines 5(2): 11-21.

Ramanathan, S. 1969. A preliminary report on Chanos fry surveys carried out in the brackishwater areas of Mannar, Puttalan and Negombo. Bull. Fish. Res. Sta. Ceylon 20: 79-85.

Schmittou, H.R. 1977. A study to determine the spawning grounds of milkfish and the environmental conditions that influence fry abundance and collection along the Antique coast of Panay Island, Philippines. In: J.W. Avault (Ed.) Proc. 8th Ann. Meet. World Maric. Soc. Louisiana State University Press, Louisiana, U.S.A.

Schuster, W.H. 1952. Fish culture in brackishwater ponds of Java. IPFC Special Publ. (1), XII. 143 pp.

Schuster, W.H. 1960. Synopsis of biological data on milkfish Chanos chanos (Forsskal), 1975. Occas. paper IPFC 59/3: 41.

Senta, T., A. Hirai, K. Kanashiro and H. Komaki. 1980. Geographical occurrence of milkfish Chanos chanos (Forsskal) fry in southern Japan. Bull. Fac. Fish. Nagasaki Univ. 48: 19-26.

Smith, I.R. 1981. The economics of milkfish fry and fingerlings industry in the Philippines. ICLARM Tec. Rep. 1. 146 pp.

Thayaparan, K. and R.D. Chakrabarty. 1984. Milkfish Aquaculture in Sri Lanka. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing, Inc., Manila, Philippines. pp. 161-169.

Thiemmedh, J. 1955. Note on the occurrence of Chanos fry in Thailand. Proc. IPFC 5th Meet. Sects. I and III. pp. 136-137.

Villaluz, A.C. 1983. Report on the milkfish and mullet juveniles in the Pacific coast of Panama. Int. Dev. Res. Center. 27 pp.

Villaluz, A.C. and A. Unggui. 1983. Effects of temperature on behavior, growth, development and survival in young milkfish, Chanos chanos (Forsskal). Aquaculture 35: 321-330.

Villaluz, A.C., H.P. Amandakoon and A. de Alwis. 1982. Milkfish fry and fingerling resources in Sri Lanka. J. Inland D. Fish. Sri Lanka 1: 7-16.

Villaluz, A.C., W.R. Villaver and R.J. Salde. 1983. Milkfish fry and fingerling industry of the Philippines: Methods and Practices. SEAFDEC Tech. Rep. No 9, 2nd Edition. p. 81.

Villaluz, D.K. 1953. Fish farming in the Philippines. Bookman, Inc., Manila, Philippines. 336 pp.

Wedemeyer, G.A. and D.J. McLeay. 1981. Methods in determining the tolerance of fishes to environmental stressors. In: A.D. Pickering (Ed.) Stress and Fish. Academic Press, Inc., Ltd., London. pp. 247-275.

7. NUTRITION AND FEEDS

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7-1. INTRODUCTION

Milkfish culture is gradually shifting from the traditional extensive aquaculture system, wherein the fish depends mainly on natural food for growth, to semi-intensive or intensive culture systems in which additional inputs such as formulated diets are used to increase fish production (Chen, 1981). This paper reviews present information on digestive organs and enzymes, food and feeding habits of the different age groups, digestibility of feedstuffs, and nutrient requirements of milkfish.

7-2. DIGESTIVE SYSTEM

The digestive tract of the milkfish consists of the mouth, pharynx, esophagus, stomach, intestine, and rectum.

The mouth is small, transverse, terminal, and toothless (Herre and Mendoza, 1929 as cited by Schuster, 1960; Chandy and George, 1960). There are four gill arches (Chandy and George, 1960) with numerous long and extremely fine gill rakers set in two diverging rows in the pharyngeal region (Schuster, 1960), suggesting that milkfish have an efficient filter-feeding apparatus. The epibranchial organs, which are elongated tubular sacs or diverticula of the pharynx located in either side of the head behind the gill cavity proper, are specialized accessory organs of the digestive system (Kapoor et al., 1975; Kafuku and Kuwatani, 1976) involved in collection or concentration and direction of food from the pharynx to the esophagus (Chandy and George, 1960; Kuwatani and Kafuku, 1978). Epibranchial organs are rudimentary with a total length of 14 mm, and completely developed in 19 mm milkfish (Kafuku and Kuwatani, 1976).

The esophagus is a straight, narrow, and somewhat flattened tube with a fairly thick wall (Chandy and George, 1960). Numerous mucous cells are found in the mucosa and a series of closely arranged mucosal folds form a spiral coil along the whole length of the esophagus. These structures aid in the rapid movement of food (Chandy, 1956). The stomach consists of two distinct parts: the cardiac stomach and the pyloric stomach. The cardiac stomach is characterized by the presence of gastric glands for both acid secretion and enzyme production (Kapoor et al., 1975; Smith, 1980) which facilitates digestion by mechanical means (Ferraris et al., 1983). The long and narrow intestine with numerous pyloric caeca at the anterior region and high degree of mucosal foldings functions in digestion and absorption (Chako, 1945; Ferraris et al., 1983).

Two other digestive organs are the liver and pancreas. The liver is a bilobed organ extending over the entire length of the esophagus with the gall bladder situated between the two lobes. The bile duct opens into the most anterior part of the intestine amidst the cluster of pyloric caeca (Chacko, 1945; Chandy and George, 1960). The pancreas, which functions in part in the production of digestive enzymes, is diffuse. Patches of pancreatic tissue are distributed in the mesentery surrounding the intestine (Chandy and George, 1960).

The digestive tract of newly-hatched larva is a simple, undifferentiated tube (Ferraris et al., 1983). In 3-day-old larvae, the esophagus, presumptive stomach, and intestine become distinguishable because of the development of mucus-secreting cells in the esophagus and the appearance of the striated border and cytoplasmic projections from the epithelial cells in the intestine. In 21-day-old fry, goblet cells start to develop in the intestine and the stomach differentiates into the cardiac and pyloric regions. The cardiac stomach appears functional in fish undergoing metamorphosis (42 days old) with the formation of the gastric glands. The development of the pyloric stomach in 21-day-old fry suggests the onset of mechanical digestion in milkfish and coincides with the time when wild fry (18-21 days old) cease their pelagic mode of life and start to enter estuarine or freshwater environments, where there is a change in feeding habits (Ferraris et al., 1983). In older fish, the structure of the digestive tract is more complex because of hyperplasia and hypertrophy of mucus-secreting and goblet cells, an increase in length of intestinal epithelium, branching of mucosal epithelium in the esophagus and intestine, presence of acellular matrix in the stomach, proliferation of gastric glands, and formation of several muscle layers (Ferraris et al., 1983). Thus, the digestive system of the milkfish is highly developed, compared to that of carps, and appears adaptable to a variety of foods.

7-3. ROLE OF VISION IN FEEDING

According to Schuster (1960), milkfish in all stages of life seek food at daytime. Banno (1980) also found that wild milkfish fry feed during daylight hours (0600 to 1900 hrs) with peak feeding at 0700 to 1300 hrs. A retinal tapetum lucidum was present in the pigment epithelium of the eye of newly-caught wild fry, which suggests that the eye is functional under subdued light conditions or in turbid environments (Kawamura and Hara, 1980; Kawamura, 1984). Kawamura and Hara (1980) demonstrated that milkfish fry depend primarily on vision during feeding. In their experiment, fry (13.7 to 13.8 mm in length) could not take brine shrimp in the dark. On the other hand, juveniles (18.9 mm in length) consumed some brine shrimp in the dark but did so less efficiently than in the light. The ability to take limited amounts of food in the dark increased with growth. This increase could be attributed to the gradual development of the chemical and auditory sense mechanisms, and not to chance encounters with food (Kawamura and Hara, 1980). Subsequent study showed that, although older milkfish (2 to 3 kg) took food day and night, significantly higher feeding activity occurred in daytime (Kawamura and Castillo, 1981).

7-4. FOOD AND FEEDING HABITS

7-4.1. IN NATURAL HABITATS

The main food ingested by adult milkfish in the wild consists of benthic and planktonic organisms (Tampi, 1958; Schuster, 1960; Poernomo, 1976; Villaluz et al., 1976; Vicencio, 1977). These include gastropods, lamellibranchs, foraminiferans, filamentous algae, diatoms, copepods and nematodes. Detritus also accounts for a large portion of the gut contents of adult fish (Tampi, 1958; Poernomo, 1976; Villaluz et al., 1976). Tampi (1958) suggested that the adult milkfish in the sea resorts to browsing for feeding.

Crear (1980) reported that adult milkfish in landlocked hypersaline ponds at Christmas Island had a higher gonado-somatic index when substantial amounts of benthic mat (composed of halophilic bacteria, blue-green algae, diatoms, and fungi) and a large population of brine shrimp (Artemia salina) were both present in the pond than when benthic mat alone was present. He also observed that reproductive readiness of the milkfish was controlled by the interaction of salinity and diet, but diet had a greater influence. It appears that brine shrimp is a desirable natural food for milkfish broodstock.

Milkfish fry and fingerlings collected along coastal areas or littoral waters fed on benthic, epiphytic and planktonic organisms which were mostly diatoms and blue-green algae, and occasionally nematodes and crustacean larvae (Tampi, 1958; Schuster, 1960; Vicencio, 1977). Moreover, wild fry (Tampi, 1958; Banno, 1980) and juveniles (Buri, 1980; Kumagai and Bagarinao, 1981) invariably consumed detritus which indicates that detritus and its associated microorganisms are an important source of nutrients for the young fish. Further study revealed that the kinds of food ingested by milkfish juveniles in the wild differed with habitat (Kumagai and Bagarinao, 1981). These results strongly suggest that milkfish can use whatever food is available in the environment.

7-4.2. IN CULTURE PONDS

The most favorable natural food for the growth of milkfish fry and fingerlings under cultivation is lab-lab (Rabanal, 1959, 1966). Lab-lab is the local term in the Philippines that denotes the biological complex composed of microbenthic plants and animals closely associated with the mud of the pond floor. The plant component consists of various forms of bacteria, unicellular and filamentous blue-green and green algae, and diatoms. The animal component consists of protozoans, copepods, ostracods, some free-living flat and round worms, molluscs in their larval stages, and some crustaceans.

There are two forms of lab-lab: floating and adhering (also referred to as attached). As the name implies, floating lab-lab is found on the surface of the pond water and is most likely lifted from the pond floor by the action of the gases produced by some components of the biological complex. The adhering lab-lab, on the other hand, is firmly attached to the pond bed.

Jumalon (1978) found some quantitative and qualitative differences between floating and adhering lab-lab. Floating lab-lab had a much lower degree of degradation; a much higher number of organisms, chlorophyll a concentration, caloric content, protein and cellulose content; and lower ash content than the adhering lab-lab. However, in terms of lipid content, there was no significant difference between the floating lab-lab, which contained 1.5% lipid (moisture-free basis), and the adhering lab-lab (1.2% lipid). Teshima et al. (1981) showed that the two types of lab-lab had similar sterol and fatty acid composition but the adhering lab-lab contained relatively higher amounts of 24-E-ethylidenecholesterol (13.3% of the sterols) than the floating lab-lab (1%). Cholesterol represented about 40% of the sterols in the two types of lab-lab. The floating and adhering lab-lab contained palmitic acid (16:0, 44, and 36%, respectively) and palmitoleic acid (16:0, 20.5, and 14%) as major fatty acids. Both types of lab-lab also contained substantial amounts of linoleic acid (18:2 ω 6, 3.6, and 6.1%, respectively) and linolenic acid (18:3 ω 6, both at 4.5%), but very low levels of eicosapentanoic acid (20:5 ω 3, 0.5 and <0.1%) and docosahexaenoic acid (22:6 ω 3, 0.5 and 0.3%). Gorriceta (1982) similarly found some amounts of 18:2 ω 6 (2.0%) and 18:3 ω 3 (4.0%) in lab-lab but no detectable amounts of 20:5 ω 3.

Although differences in the floating and adhering lab-lab do exist, milkfish in ponds either feed closely to the surface or take food from the bottom (Schuster, 1960), indicating that the fish have no distinct preference for taking food from either floating or adhering lab-lab.

Earlier analysis of gut contents of milkfish in ponds showed that fish measuring 55 to 115 mm in length ingested plant items (diatoms, blue-green algae, filamentous green algae), nematodes and crustacean eggs. Larger fish (116 to 402 mm in length) fed on larvae and eggs of copepods, polychaetes and ostracods in addition to plant materials (Hiatt, 1944, cited by Tampi, 1958 and Schuster, 1960). Milkfish measuring 200 to 300 mm in length fed preferably on soft benthic blue-green algae and diatoms in ponds, but when these were insufficient, the fish took decayed filamentous green algae and other higher aquatic plants (Schuster, 1949, cited by Schuster, 1960). Subsequent study also showed that, of the four major algal groups, benthic diatoms and blue-green algae were the most desirable food for all age groups of milkfish in brackishwater ponds (Tang and Hwang, 1966). Like wild fish, milkfish fingerlings (5 to 15 cm in length) fed more on phytoplankton than on zooplankton while larger fish (16 to 60 cm) consumed filamentous green algae in addition to the plankton (Vicencio, 1977). Similar results were obtained by Esguerra (1951). However, it was demonstrated that milkfish (350 mm in length) lost weight when fed exclusively with fresh filamentous green algae (Chaetomorpha) in aquaria (Schuster, 1949, cited by Schuster, 1960). The filamentous green algae, as well as other higher aquatic plants, become suitable food for the milkfish only when they are in a partially decayed state because they are soft and easily macerated (Schuster, 1960). The fish probably also benefit from the microorganisms growing on the decayed material.

Thus, milkfish can be considered planktonic, epiphytic, benthic or microphagous herbivorous feeders. This is because the milkfish resort to faculative feeding (Schuster, 1960) depending on the availability of food.

7-4.3. UNDER LABORATORY CONDITIONS

Laboratory experiments have shown that some foods have higher nutritional value than others. Pantastico et al. (1983) found that *Oscillatoria* alone or in combination with *Chroococcus* resulted in higher survival and growth rates compared to *Navicula* alone. Feeding the fry with ^{14}C-labeled algae showed that *Oscillatoria* alone and *Chroococcus* alone were assimilated at higher rates while negligible amounts of *Navicula*, *Chlorella*, and *Euglena* were assimilated.

Carreon et al. (1984) reported that growth and survival were high for wild milkfish fry supplied with natural plankton, but low for those reared on artificial detritus. Natural plankton was produced by fertilizing impounded tap water with chicken manure and complete inorganic fertilizer, and contained a mixture of phytoplankton, (*Microcystis*, *Scenedesmus*, and diatoms), and zooplankton (ostracods, cladocerans, and copepods). Artificial detritus was made from ground rice straw and hulls combined with powdered chicken manure and aged in water 10 to 15 days with aeration. Although detritus is the major food ingested by milkfish fry (Banno, 1980) and juveniles (Buri, 1980; Kumagai and Bagarinao, 1981) in their natural habitat, the type and quantity of detritus may have an influence on its acceptability and value as food for milkfish.

7-5. DIGESTIVE ENZYMES

Table 1 presents the enzymes for carbohydrate, protein and lipid digestion detected in the crude extracts from the various parts of the milkfish digestive system. Among the carbohydrases, those involved in the hydrolosis of α-glucosidic bonds were most active (Chiu and Benitez, 1981). Intestinal amylase activity always peaked at about noon (1230

Table 1. Digestive enzymes detected in crude extracts from various parts of the milkfish digestive system.

Enzyme	Optimum pH & temperature	References & remarks
I. Carbohydrates		Chiu & Benitez (1981). Fish (35 g) were grown on unicellular algae or filamentous green algae for one to six months.
a. amylase	pH 6.2, 50°C	optimum pH and temperature for intestinal amylase; high activity in pancreas, intestines, pyloric caeca & liver
b. maltase, trehalase & dextrinase		detected in intestines & pyloric caeca
c. β-glucosidase & β-galactosidase		with limited substrate specificity; detected in intestines & pyloric caeca
II. Proteases		Benitez & Tiro (1982); detected in esophagus, liver, pyloric caeca, intestines, and pancreas
a. intestinal protease	pH 7.2 & 9.3, 50 to 60°C	for fish (223 g) grown on unicellular algae
	pH 10; 45-60°C	for fish (160 g) grown on filamentous green algae
b. trypsin		for fish grown on unicellular algae; detected in intestines, pyloric caeca, & pancreas
c. chymotrypsin		for fish grown on unicellular algae and filamentous green algae; detected in intestines, pyloric caeca & pancreas
d. pepsin	pH 2	detected in stomach
III. Lipases		Gorriceta (1982); Gorriceta & Benitez (1983). Fish size, 240 g; detected in esophagus, epibranchial organs, stomach, intestine, pyloric caeca, liver & pancreas
a. intestinal lipase	pH 8 & 6.8; 45°C	optimum pH and temperature for anterior intestinal lipase
b. pancreatic lipase	pH 8.6 & 6.4; 50°C	

hrs) when the gut was full and was lowest at 0030 hrs when the gut was empty. This is consistent with earlier reports that milkfish are daytime feeders and suggests that amylase secretion in milkfish is in phase with feeding activity (Chiu and Benitez, 1981). Carbohydrases of lower activity and limited specificity in milkfish include β-glucosidase and β-galactosidase. Presence of lactase (a β-galactosidase) and α-galactosidase was not evident. Although the milkfish fed on algae and higher aquatic plants, cellulose activity could not be detected in any region of the digestive tract (Chiu and Benitez, 1981). Thus, while glycogen and starch are readily digestible, cellulose in plant materials is indigestible to the milkfish.

Benitez and Tiro (1982) observed that protease activity was higher in milkfish that were grown in ponds containing unicellular algae than those grown in ponds containing filamentous green algae, Chaetomorpha, as the predominant natural food. In both groups of fish, high protease activity occurred in crude extracts from the pyloric caeca, intestines and pancreas. The presence of two alkaline proteases, trypsin and chymotrypsin, was detected in the crude extracts of milkfish that fed on unicellular algae. However, only chymotrypsin was detected in fish that fed on the filamentous green algae, Chaetomorpha. The apparent absence of trypsin activity in the crude extracts was caused by a powerful trypsin inhibitor found in Chaetomorpha (Benitez and Tiro, 1982). The presence of a trypsin inhibitor may partly explain the weight loss of milkfish fed exclusively with Chaetomorpha (Schuster, 1949, cited by Schuster, 1960) and the slow growth of milkfish in ponds where the algae predominate (Benitez and Tiro, 1982; Benitez, 1984).

The pepsin activity observed at pH 2 in the stomach extracts of milkfish (Benitez and Tiro, 1982) is attributed to the gastric glands in the stomach which function in hydrochloric acid secretion and enzyme production (Ferraris et al., 1983). Presumably, protein digestion starts in the stomach soon after the development of the gastric glands in about 42-day-old milkfish.

Histochemically, aminopeptidase was found localized in the striated border of the intestinal epithelium of 21-day-old fry and older fish (Ferraris et al., 1983). This indicates that the milkfish intestine is capable of enzyme secretion.

The distribution pattern of lipases in the digestive tract of milkfish has been determined (Gorriceta, 1982; Gorriceta & Benitez, 1983). Although lipase activity was detected in almost all parts of the digestive tract, the intestines, pancreas and pyloric caeca were the major sites of lipase secretion. Extracts obtained from milkfish grown in ponds containing lab-lab showed relatively higher lipase activity than those from milkfish grown in ponds containing filamentous green algae as the main natural food. In both groups of fish, lipase activity was higher at the anterior intestine than at the posterior intestine.

Optimum temperatures for enzyme activity determined _in vitro_ were rather high (Table 1). Consequently, within physiological limits, digestive enzymes in milkfish are expected to be more active during warmer months when ambient water temperatures are higher. This supports common observation that growth rates and feeding activity of milkfish in the cold months are lower than in the warm months (Benitez, 1984).

Other enzymes involved in lipid digestion and synthesis were found by histochemical techniques (Ferraris et al., 1983). Alkaline phosphatase was located in the striated border of the intestine of the milkfish fry and older fish. Nonspecific carboxylic esterases were diffusely distributed in the cytoplasm of the intestinal epithelial cells from the early fry stage to adult stage. These findings further show that the milkfish intestine also functions in enzyme secretion.

Obviously, the major enzymes for lipid, protein and carbohydrate digestion are present in milkfish. However, other digestive enzymes must be characterized and their development in younger fish determined.

7-6. DIGESTIBILITY OF FEEDSTUFFS

Data on protein digestibility of several feedstuffs by milkfish as determined by Ferraris et al. (1984) are presented in Table 2. Gelatin had the highest digestibility coefficient regardless of fish size. Digestibility of casein, defatted soybean meal and fish meal increased with fish size in fresh water but not in sea water. Leucaena

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Table 2. Protein digestibility of feedstuffs by different size groups of milkfish in freshwater (F) and sea water (S)*

Feedstuff		Digestibility Coefficent (%)		
		2 g	60 g	175 g
Casein	F	58	84	88
	S	73	49	65
Gelatin	F	95	94	98
	S	96	98	97
Soybean meal	F	54	69	95
(defatted)	S	76	54	58
Fish meal	F	44	70	73
	S	71	52	71
Ipil-ipil	F	47	40	42
	S	61	31	**

* Source: Ferraris et al. (1984)

** Unrealistic value of -10.

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leucocephala (ipil-ipil) leaf meal was the least digestible. More studies of this issue must be conducted.

7-7. NUTRIENT REQUIREMENTS

Lee and Liao (1976) first developed a purified diet for milkfish nutritional studies. Vitamin-free casein (about 60% of the diet) supplemented with 0.5% L-tryptophan was a better protein source than a combination of casein and gelatin for the young milkfish (1.7 g body weight). Ten percent soybean oil was better as a lipid source than shark liver oil. Vitamin and mineral mixtures (4 and 10% of the diet, respectively) recommended for chinook salmon (Halver, 1957) were found satisfactory for milkfish. This purified diet also contained dextrin as a carbohydrate source and a high level of carboxymethyl cellulose (10%) as a binder. Recently, Teshima et al. (1984) also developed purified diets for milkfish fingerlings under laboratory conditions. They found that high growth occurred when a diet containing 35% casein and 15% gelatin supplemented with 0.5% methionine and 0.5% tryptophan was fed at 30 to 50% of fish biomass twice daily.

A study showed that wild milkfish fry (40 mg body weight) required 40% dietary protein for maximum growth, efficient conversion and high survival rate (Lim et al., 1979). The experiment was done under laboratory conditions; salinity ranged from 32 to 34 ppt and temperature from 25 to 28°C. The test diets, fed at 10% of fish biomass daily, contained casein as protein source, dextrin as carbohydrate source, equal parts of cod liver oil and corn oil as lipid sources, and vitamin and mineral premixes. The protein requirement of milkfish fry is near the values reported for other warmwater fishes, particularly *Tilapia mossambica* (Jauncey, 1982) and *Cyprinus carpio* (Ogino and Saito, 1970). Further study on protein-energy requirement showed that 30 to 40% dietary protein, 10% lipid, and 25% carbohydrate were required by milkfish fingerlings weighing 0.5 to 0 8 g (Pascual, 1984).

The amino acid composition of precipitable proteins from the whole body of milkfish fry (7.1 mg initial weight) reared on Artemia salina nauplii and Brachionus sp. was determined at weekly intervals and a reference amino acid pattern was calculated (Coloso et al., 1983). The amino acid contents of the fry at different time intervals were similar. Because lysine, leucine and arginine occurred at high proportions in the pattern, they are most likely to be the first limiting amino acids (Coloso et al., 1983). The reference amino acid pattern could be useful in the formation of test diets for quantifying essential amino acid requirements of the milkfish. This pattern could also be an indirect basis for estimating milkfish requirements for the essential amino acids as long as the quantitative requirement for at least one essential amino acid is known (Cowey and Tacon, 1981; Jauncey et al., 1983; Santiago, 1985).

The dietary requirement of milkfish fingerlings (0.83 g mean body weight) for lipid (cod liver oil) is about 7 to 10% (Alava and de la Cruz, 1983). This is similar to recommended dietary levels of 8 to 10% lipid from corn oil and cod liver oil (1:1 ratio) for milkfish fry (Camacho and Bien, 1983). Histology of the milkfish livers showed that cellular and structural integrity was maintained when diets contained about 7 to 10% dietary lipid (Alava and de la Cruz, 1983). Lipid levels lower than 7% resulted in decreased granulation and loss of nuclei of liver cells. Dietary lipid exceeding 10% caused minor disruption of hepatocytes due to formation of large lipid vacuoles, loss of hepatic cord with development of fibrous tissues, and occurrence of pyknotic nuclei (Alava and de la Cruz, 1983).

With the use of purified test diets, Bautista and de la Cruz (1983) found that growth and survival of milkfish (1.6 g body weight) fed diets containing linoleic and linolenic acids were significantly higher than those of fish fed lipid-free diets or diets with 7% lauric acid as lipid source. Although highest growth was attained by fish fed a diet with 1% linolenic acid, growth and survival of fish fed with linoleic and linolenic acids were not significantly different. Fatty acid analysis revealed that the lipid-free diet and the diet with lauric acid increased the levels of monoenoic acids in the fish while diets with linoleic and linolenic acids decreased the monoenes and increased the levels of long-chained polyunsaturated fatty acids. Results suggest that both linoleic and linolenic acids are effective in promoting high growth and survival rates in milkfish (Bautista and de la Cruz, 1983). Hence, cod liver oil (high in linolenic acid) and corn oil (high in linoleic acid) are good sources of lipids for milkfish fry and fingerlings. Although there are indications that milkfish have the ability to use linoleic and linolenic acids as precursors for the biosynthesis of long-chain polyunsaturated fatty acids of $\omega 6$ and $\omega 3$ series (Gorriceta, 1982; Villegas et al., 1983), it remains to be shown whether long-chain polyunsaturated fatty acids have better growth-enhancing effects than the linoleic and/or linolenic acids. Optimum levels of these essential fatty acids in milkfish diets have yet to be determined.

Very little is known about the vitamin and mineral requirements of the milkfish. Nevertheless, various vitamin and mineral premixes, such as those intended for the coldwater fish, chinook salmon (Halver, 1957), and for other warmwater fishes (NRC, 1977), have been used for milkfish (Lee and Liao, 1976; Lim et al., 1979; Santiago et al., 1983; Piedad-Pascual, 1983) with satisfactory results.

Practically nothing is known about the nutrient requirements of milkfish broodstock and larvae. Hence, further studies along this line are needed.

7-8. ARTIFICIAL DIETS

Using available information on the nutrient requirements of the milkfish and other warmwater fishes (NRC, 1977; 1983), formulation and evaluation of artificial diets for milkfish were initiated. Feeding wild milkfish fry in net enclosures in ponds with formulated diets containing 20, 30, 40 or 50% crude protein, with or without vitamin supplementation, showed that the 40% protein diet produced maximum growth (Seraspe, 1979). This confirms the findings of Lim et al. (1982). Furthermore, survival rate was higher with the vitamin-enriched diet than with the incomplete diet. In a subsequent study, practical diets containing 30 to 40% crude protein with fish meal and full-fat soybean meal as major protein sources produced a significant increase in survival rate of milkfish fingerlings (2.8 g body weight) as the vitamin supplement increased from 4 to 6% of the diets (Piedad-Pascual, 1983).

Various feedstuffs--fish meal, meat and bone meal, shrimp head meal, soybean meal, copra meal and Leucaena leucocephala (ipil ipil) leaf meal--were tested as protein sources in formulated diets fed to milkfish fingerlings weighing 0.67 g (Samsi, 1979). Animal protein sources were better utilized than plant protein sources. Among the three animal feedstuffs, fish meal and meat and bone meal gave significantly high mean weight gains, although there were no significant differences in survival rates. Among the plant feedstuffs, only soybean meal gave acceptable growth and survival rates. These results could partly explain earlier observation that young milkfish fed with high-protein trout pellets based on fish meal had higher weight gain and survival rate than those fed with low-protein rabbit pellets containing basically alfalfa (Timbol, 1969).

Other practical diets have been evaluated as feeds for young milkfish in freshwater and seawater environments.

Milkfish fry (15 mg body weight) fed four formulated dry diets containing 40% crude protein with fish meal as the major protein source had significantly higher survival rates and weight gains compared to those given *Moina* sp.(a cladoceran) and blended water hyacinth leaves (Santiago et al., 1983). The fry had some difficulty feeding on *Moina* sp. which were too active and seemed too large for the fry. The blended water hyacinth had low macronutrient content which explains the low survival and growth of the fry fed with it. In another study, six practical diets containing about 42% crude protein from varying amounts of shrimp head meal, soybean meal, meat and bone meal, corn gluten meal, rice bran and wheat flour were fed to the milkfish fry (9 mg initial weight). All diets gave high survival rates, growth and protein efficiency ratio (Lim and Alava, 1983).

Variable results were obtained when artificial diets were fed to milkfish in ponds. Fish pellets (37.4% crude protein) considerably increased fish production compared to lab-lab or plankton as the main food for the milkfish (Fortes, 1984). On the other hand, commercial chick starter pellets (21% crude protein) used as a supplemental diet for milkfish fingerlings (16.3 g body weight) did not significantly increase growth, survival and production of milkfish in fertilized ponds (Otubusin and Lim, 1985). Undoubtedly, the effectiveness of an artificial diet in enhancing fish production is influenced by factors such as the nutritional quality of the diet, stocking rate or fish biomass, and the level of fertilization or natural food production, among others.

Studies on feeding of milkfish broodstock with artificial diets are meager. Commercial fish diets (Lee, 1983; Marte et al., 1983) and formulated diets (Liao and Chen, 1979) have been used in the development and maintenance of milkfish broodstock but effects of different diets on reproductive performance of the milkfish have not been evaluated. Broodstock diets that hasten gonadal maturation or rematuration of milkfish for successful induced spawning efforts now in progress have to be developed.

A shift from natural food to an artificial diet has been shown feasible in hatchery-bred milkfish larvae (Duray and Bagarinao, 1984). Fifteen-day-old larvae, which were reared on rotifers prior to the experiment, were fed six commercial and experimental diets with Artemia nauplii as a control. Survival rates of the larvae fed with the artificial diets were comparable to, or significantly higher than, the survival rates of larvae fed with Artemia nauplii. Although mean body weight was highest for larvae fed with Artemia, mean condition factors and standard lengths were not significantly different among treatments. Therefore, milkfish can respond positively to artificial diets from an early fry stage to adult stage. However, the nutritional quality of existing diets needs further improvement.

7-9. RECOMMENDATIONS

Clearly, a good deal is known regarding the natural food needed to rear milkfish at different life stages and the use of natural food will continue to be important in milkfish culture for some time. Thus, researches on growth enhancement of desirable species of natural food in the culture system are useful. Factors that influence variation of natural food species from pond to pond have to be studied. In the long term, artificial diets will be the principal food in growing young milkfish to marketable or adult size and in the management of milkfish broodstock. Although practical diets for milkfish can now be developed, further studies have to be done to improve the nutritional quality of these diets. Important research areas include nutrient requirements and metabolism, digestive enzymes, digestibility of feedstuffs, and other aspects of digestive physiology, with emphasis on milkfish broodstock and larvae.

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REFERENCES

Acosta, B. O. and J. V. Juario. 1983. Biological evaluation of Brachionus plicatilis fed Chlorella sp., Isochrysis galbana and Tetraselmis sp. and their combinations as feed for milkfish (Chanos chanos Forsskal) fry. Poster paper presented during the Second International Milkfish Aquaculture Conference, Iloilo City, Philippines. Oct. 4-8, 1983.

Alava, V. R. and M. C. de la Cruz. 1983. Quantitive dietary fat requirement of Chanos chanos fingerlings in a controlled environment. Paper presented during the Second International Milkfish Aquaculture Conference. Iloilo City, Philippines. Oct. 4-8, 1983.

Banno, J. E. 1930. The food and feeding habit of the milkfish fry Chanos chanos (Forsskal) collected from the habitats along the coast of Hamtik, Antique. MS. thesis. University of the Philippines in the Visayas, Ilolio. 77 pp.

Bautista, M. N. and M. C. de la Cruz. 1983. Effects of dietary linoleic and linolenic acids on growth, survival, fatty acid composition, and liver histology of milkfish fingerlings. Paper presented during the Second Internation Milkfish Aquaculture Conference, Iloilo City. Oct. 4-8, 1983.

Benitez, L. V. 1984. Milkfish nutrition. In: J. V. Juario, R. P. Ferraris, and L. V. Benitez (Eds.). Advances in milkfish biology and culture. Island Publishing House, Inc. Metro Manila, Philippines. pp. 133-143.

Benitez, L. V. and L. B. Tiro. 1982. Studies on the digestive proteases of the milkfish, Chanos chanos. Mar. Biol. 71: 309-315.

Buri, P. 1980. Ecology on the feeding of milkfish fry and juveniles, Chanos chanos (Forsskal) in the Philippines. Mem. Kagoshima Univ. Res. Center S. Pac. 1: 25-42.

Camacho, A. S. and N. Bien. 1983. Studies on the nutrient requirements of milkfish Chanos chanos (Forsskal). Paper presented at the Technical Symposium on Aquaculture, University of the Philippines in the Visayas, Feb. 19, 1983. 17 p. (mimeo).

Carreon, J. A., L. V. Laureta, F. A. Estocapio, and T. U. Abalos. 1984. Milkfish seedling survival in raceways of freshwater recirculating systems. Aquaculture, 36: 257-272.

Chacko, P. I. 1945. On the food and alimentary canal of the milkfish Chanos chanos (Forsskal). Curr. Sci., 14: 242-243.

Chandy, M. 1956. On the oesophagus of the milkfish Chanos chanos (Forsskal). J. Zool. Sci. India. 8(1): 79-84.

Chandy, M. and M. G. George. 1960. Further studies on the alimentary tract of milkfish Chanos in relation to its food and feeding habits. Proc. Nat. Inst. Sci. India, 26(B3): 126-134.

Chen, T. P. 1981. Taiwan farmers go deep for milkfish. Fish Farming International, 8: 12-14.

Chiu, Y. N. and L. V. Benitez. 1981. Studies on the carbohydrases in the digestive tract of the milkfish Chanos chanos. Mar. Biol. 61: 247-254.

Coloso, R.M., L.B. Tiro and L.V. Benitez. 1983. A reference amino acid pattern for milkfish, Chanos chanos juveniles. Paper presented during the Second International Milkfish Aquaculture Conference. Iloilo City, Philippines. Oct. 4-8, 1983.

Cowey, C. B. and A. G. J. Tacon. 1981. Fish nutrition--relevance to invertebrates. Proc. Second International Conference on Aquaculture Nutrition. Biochemical Approaches to Shellfish Nutrition. Univ. of Delaware. Oct. 27-29, 1981. pp. 13-29.

Crear, D. 1980. Observations on the reproductive state of milkfish populations (Chanos chanos) from hypersaline ponds on Christmas Island (Pacific Ocean). Proc. World Maricul. Soc. 11: 548-556.

Duray, M. and T. Bagarinao. 1984. Weaning of hatchery-bred milkfish larvae from live food to artificial diets. Aquaculture, 41: 325-332.

Esguerra, R. S. 1951. Enumeration of algae in Philippine bangus fishponds and in the digestive tract of the fish with notes on conditions favorable for their growth. Philipp. J. Fish. 1: 175-196.

Ferraris, R. P., J. D. Tan and M. C. de la Cruz. 1983. The developmental morphology of the digestive tract in the milkfish Chanos chanos Forsskal. Paper presented during the Second International Milkfish Aquaculture Conference. Ilolio City, Philippines. Oct. 4-8, 1983.

Ferraris, J. P., M. R. Catacutan, R. L. Mabelin, and A. P. Jazul. 1984. Effect of fish size and salinity on intestinal passage time and protein digestibility of feedstuffs in milkfish Chanos chanos. Terminal report submitted to SEAFDEC AQD.

Fortes, R. D. 1984. Milkfish culture techniques generated and developed by the Brackishwater Aquaculture Center. In: J. V. Juario, R. P. Ferraris and L. V. Benitez (Eds.). Advances in milkfish biology and culture. Island Publishing House, Inc., Metro Manila, Philippines. pp. 107-199.

Gorriceta, I. R. 1982. Studies on the digestive lipases and lipid composition of milkfish, Chanos chanos Forsskal. M. S. Thesis, Univ. of the Philippines System. 56 pp.

Gorriceta, I. R. and L. V. Benitez. 1983. Lipid digestion in milkfish grown in ponds on two types of natural food bases. Paper presented during the Second International Milkfish Aquaculture Conference. Ilolio City, Philippines. Oct. 4-8, 1983.

Halver, J. E. 1957. Nutrition of salmonid fishes III. Water-soluble vitamin and mineral requirements of chinook salmon. J. Nutr. 62: 225-243.

Herre, A. W. and J. Mendoza. 1929. Bangos culture in the Philippine Islands. Philipp. J. Sci. 38(4): 451-509. (Cited in Schuster, 1960).

Hiatt, R. W. 1944. Food chains and the food cycle in Hawaiian fish ponds. Part I. The food and feeding habits of mullet (Mugil cephalus), milkfish (Chanos chanos), and the ten pounder (Elops machnata). Trans. Amer. Fish. Soc. 74: 250-261. (Cited in Tampi, 1958 and Schuster, 1960).

Jauncey, K., A. G. J. Tacon and A. J. Jackson. 1983. The quantitative essential amino acid requirements of Oreochromis (=Sarotherodon) mossambicus. In: L. Fishelson and Z. Yaron (compilers). International Symposium on Tilapia in Aquaculture. (1st 1983: Nazareth, Israel) Proc. Tel Aviv University, Israel.

Jauncey, K. 1982. The effects of dietary protein level on the growth, feed conversion, protein utilization and body composition of juvenile tilapias (Sarotherodon mossambicus). Aquaculture 27: 43-54.

Jumalon, N. A. 1978. Selection and application of a suitable sampling method for quantitative and qualitative evaluation of lab-lab. M. S. thesis. University of the Philippines. 121 pp.

Kafuku, T. and Y. Kuwatani. 1976. Physiological functions of the epibranchial organ of milkfish from the point of its ontogeny. International Milkfish Workshop Conference. May 19-22, 1976. SEAFDEC-IDRC Tigbauan, Iloilo, Philippines. pp. 47-49.

Kapoor, B. G., H. Smith and I. A. Verighina. 1975. The alimentary canal and digestion in teleost. In: F. S. Russel and M. Yonge (eds.). Adv. Mar. Biol. 13: 109-239.

Kawamura, G. 1984. The sense organs and behavior of milkfish fry in relation to collection techniques. In: J. V. Juario, R. P. Ferraris and L. V. Benitez (Eds.). Advances in Milkfish Biology and Culture. Island Publishing House, Inc., Metro Manila, Philippines. pp. 69-84.

Kawamura, G. and S. Hara. 1980. On the visual feeding of milkfish larvae and juveniles in captivity. Bull. Jap. Soc. Sci. Fish. 46: 1297-1300.

Kawamura, G. and A. R. Castillo, Jr. 1981. A new device for recording the feeding activity of milkfish. Bull. Jap. Soc. Sci. Fish. 47(1): 141.

Kumagai, S. and T. U. Bagarinao. 1981. Studies on the habitat and food of juvenile milkfish in the wild. Fish. Res. J. Philipp. 6: 1-10.

Kuwatani, Y. and T. Kafuku. 1978. Morphology and function of epibranchial organ studied and inferred on milkfish. Bull. Freshwater Fish. Res. Lab., Tokyo. 28: 221-236.

Lee, C. S. 1983. Preliminary studies on the maturation of milkfish Chanos chanos Forsskal, in an environmentally controlled system. Paper presented during the Second International Milkfish Aquaculture Conference. Ilolio City, Philippines. Oct. 4-8, 1983.

Lee, D. L. and I. C. Liao. 1976. A preliminary study on the purified test diet for young milkfish, Chanos chanos. Proc. International Milkfish Workshop Conference. Tigbauan, Ilolio, Philippines. May 19-22, 1976. pp. 104-120.

Liao, I. C. and T. I. Chen. 1979. Report on the induced maturation and ovulation of milkfish (Chanos chanos) reared in tanks. Proc. World Maricul. Soc. 10: 317-331.

Lim, C. and V. R. Alava. 1983. Artificial diets for Chanos chanos (Forsskal) fry. Poster paper presented during the Second International Milkfish Aquaculture Conference. Ilolio City, Philippines. Oct. 4-8, 1983.

Lim, C., S. Sukhawongs, and F. P. Pascual. 1979. A preliminary study on the protein requirement of Chanos chanos (Forsskal) in a controlled environment. Aquaculture, 17: 195-201.

Lim, C.L., Y.Y. Ting, and Y.L. Song. 1982. Proceeding evaluation of HIVAX Vibrio anguillarum bacterin in the vaccination of milkfish (Chanos chanos) fingerlings. CAPD Fish. Ser. No. 8, Fish Disease Research 4: 80-83.

Marte, C. L., F. J. Lacanilao, and J. V. Juario. 1983. Completion of the life cycle of milkfish Chanos chanos (Forsskal) in captivity. Paper presented during the Second International Milkfish Aquaculture Conference. Ilolio City, Philippines. Oct. 4-8, 1983.

National Research Council (NRC). 1977. Nutrient requirements of warmwater fishes. National Academy of Sciences. Washington, D. C. 78 pp.

Ogino, C. and K. Saito. 1970. Protein nutrition in fish. I. The utilization of dietary protein by young carp Bull. Jap. Soc. Sci. Fish. 36: 250-254.

Otubusin, S.O. and C. Lim. 1985. The effect of duration of feeding on survival, growth and production of milkfish, Chanos chanos (Forskal) in brackishwater ponds in the Philippines. Aquaculture 46: 287-292.

Pantastico, J. B., J. P. Baldia and D. M. Reyes. 1983. Feed preference of milkfish (Chanos chanos Forsskal) fry given different algal species as natural feed. Paper presented during the Second International Milkfish Aquaculture Conference. Ilolio City, Philippines. Oct. 4-8, 1983.

Pascual, F. P. 1984. The energy-protein requirement of Chanos chanos fingerlings. Poster paper presented during the International Symposium on Feeding and Nutrition in Fish. Univ. of Aberdeen. July 10-13, 1984.

Piedad-Pascual, F. 1983. Biological evaluation of some practical diets developed for milkfish fingerlings. Poster paper presented during the Second International Milkfish Aquaculture Conference. Ilolio City, Philippines. Oct. 4-8, 1983.

Poernomo, A. 1976. Notes on food and feeding habits of milkfish (Chanos chanos) from the sea. Proc. International Milkfish Workshop Conference. May 19-22, 1976. Tigbauan, Ilolio. SEAFDEC-IDRC. pp. 162-166.

Rabanal, H. R. 1959. The culture of lab-lab, the natural food of the milkfish or bangos, Chanos chanos (Forsskal) fry and fingerlings under cultivation. Fisheries Gazette. 3(2): 12-18.

Rabanal, H. R. 1966. The culture of lab-lab, the natural food of milkfish or bangos, Chanos chanos (Forsskal) fry and fingerlings under cultivation. Phil. Fish. J. 35: 22-26.

Samsi, S. 1979. Effects of various protein sources on the growth and survival rates of milkfish (Chanos chanos Forsskal) fingerlings in a controlled environment. MS. thesis. Univ. of the Philippines in the Visayas. 42 pp.

Santiago, C. B. 1985. Amino acid requirements of Nile tilapia. Ph.D. dissertation. Auburn University, Alabama. 141 pp.

Santiago, C. B., M. B. Aldaba, and E. T. Songalia. 1983. Effect of artificial diets on growth and survival of milkfish fry in fresh water. Aquaculture 34: 247-252.

Schuster, W. H. 1960. Synopsis of biological data on milkfish Chanos chanos (Forsskal), 1775. FAO Fisheries Biology Synopsis No. 4. Fisheries Division, Biology Branch. FAO, Rome. 60 pp.

Schuster, W.H. 1949. On the food of bandeng, Chanos chanos Forskal. Commun. Gen. Agric. Res. Sta. Bagor. Vol. 82:20 (cited in Schuster, 1960).

Seraspe, E. B. 1979. Intensive feeding of milkfish fry (Chanos chanos Forsskal) in net enclosures using complete and incomplete diets at various protein levels. M.S. thesis. Univ. of the Philippines in the Visayas. 61 pp.

Smith, L. S. 1980. Digestion in teleost fishes. In: Fish Feed Technology. UNDP/FAO Publication ADCP/REP/80/11. Rome. p. 3-18.

Tampi, P. R. S. 1958. On the food of Chanos chanos (Forsskal). Indian J. Fish. 5: 107-117.

Tang, T. and T. Hwang. 1966. Evaluation of the relative suitability of various groups of algae as food of milkfish produced in brackishwater ponds. In: T. V. R. Pillay (Ed.). Proc. of the FAO World Symposium on Warm-Water Pond Fish Culture. FAO Fisheries Reports No. 44, 3: 365-372.

Teshima, S., A. Kanazawa and G. Kawamura. 1984. Effects of several factors on growth of milkfish (Chanos chanos Forsskal) fingerlings reared with artificial diets in aquaria. Aquaculture 37: 39-50.

Teshima, S., A. Kanazawa and A. Tago. 1981. Sterols and fatty acids of the lab-lab and snail from the milkfish pond. Mem. Fac. Fish. Kagoshima Univ. 30: 317-323.

Timbol, A. S. 1969. The growth of young milkfish, Chanos chanos Forsskal, on two types of pelleted food. University of Hawaii, Honolulu. 45 pp.

Vicencio, Z. T. 1977. Studies on the food habits of milkfish Chanos chanos (Forsskal). Fish. Res. J. Phil. 2: 3-18.

Villaluz, A. C., L. B. Tiro, L. M. Ver and W. E. Vanstone. 1976. Qualitative analysis of the contents of the anterior portion of the oesophagus from adult milkfish, Chanos chanos, captured in Pandan Bay from May 10-June 16, 1975. Proc. International Milkfish Workshop Conference. Tigbauan, Iloilo, Philippines. May 19-22, 1976. pp. 228-231.

Villegas, C. T., O. M. Millamena and F. Escritor. 1983. Growth, survival and fatty acid composition of Chanos chanos fry fed Brachionus plicatilis reared on three selected algal diets. Paper presented during the Second International Milkfish Aquaculture Conference. Iloilo City, Philippines. Oct. 4-8. 1983.

8. MILKFISH CULTURE METHODS IN SOUTHEAST ASIA*

by

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8-1. INTRODUCTION

Milkfish culture practices have a long history in Southeast Asia. Although little is known about the origin of milkfish farming, it is generally believed to have begun in Indonesia, where fish farming in saltwater ponds dates back at least 500 years (Schuster, 1952). It was introduced to the Philippines and Taiwan in the 16th century. In Taiwan, milkfish farming was already practiced during the reign of Gen. Cheng Cheng-Kung (Koxinga) (1661-1683 A.D.). At that time, many milkfish farms were built in Annan Section of Tainan City (Chen, 1976). The industry has grown over the past 300 years and now almost 32,000 tons of milkfish are

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* Contribution B No. 36 from the Tungkang Marine Laboratory

produced annually from 16,000 ha, or 18.88% of Taiwan's total aquaculture production (Fig. 1) (Taiwan Fisheries Bureau, 1983). In the Philippines, milkfish is the major culture species, and the annual yields from freshwater fish pens and brackishwater ponds are increasing steadily (Samson, 1984). In Indonesia, milkfish is esteemed as a high-value food fish. The government assists farmers in improving production and sets a target yield of 800-1,000 kg/ha/yr (Chong et al., 1984).

Although milkfish culture is not a young industry in Southeast Asia, many constraints and problems remain. In this chapter, an overview is made of the milkfish fry industry, pond design and construction, culture methods and management, as well as marketing and processing. Included is recent technological progress from work conducted in Taiwan, the Philippines and Indonesia. Problems, prospects and recommendations are also presented.

8-2. FRY INDUSTRY

Fry catch fluctuates annually because of variations in meteorological, oceanographical and biological factors. Fishing efforts also affect the number of fry caught. In Taiwan from 1965 to 1983, the catch varied from a low of 33.96 million (1967) to a high of 234.87 million (1970). Since 1970, fry catch has decreased yearly to about 103.532 million in 1983 (Taiwan Fisheries Bureau, 1983). Current fry catches cannot meet the demands of the milkfish industry in Taiwan (Fig. 1).

Similar methods and practices are used in Southeast Asian countries to collect the milkfish fry and fingerlings, with some modifications in different areas (see Chapter 6).

With a long culture history, Tainan county is the fry trade, nursery and growout center of Taiwan. Seventy percent of the fry and 47% of the total milkfish culture area are located here. The structure of the milkfish system in Taiwan

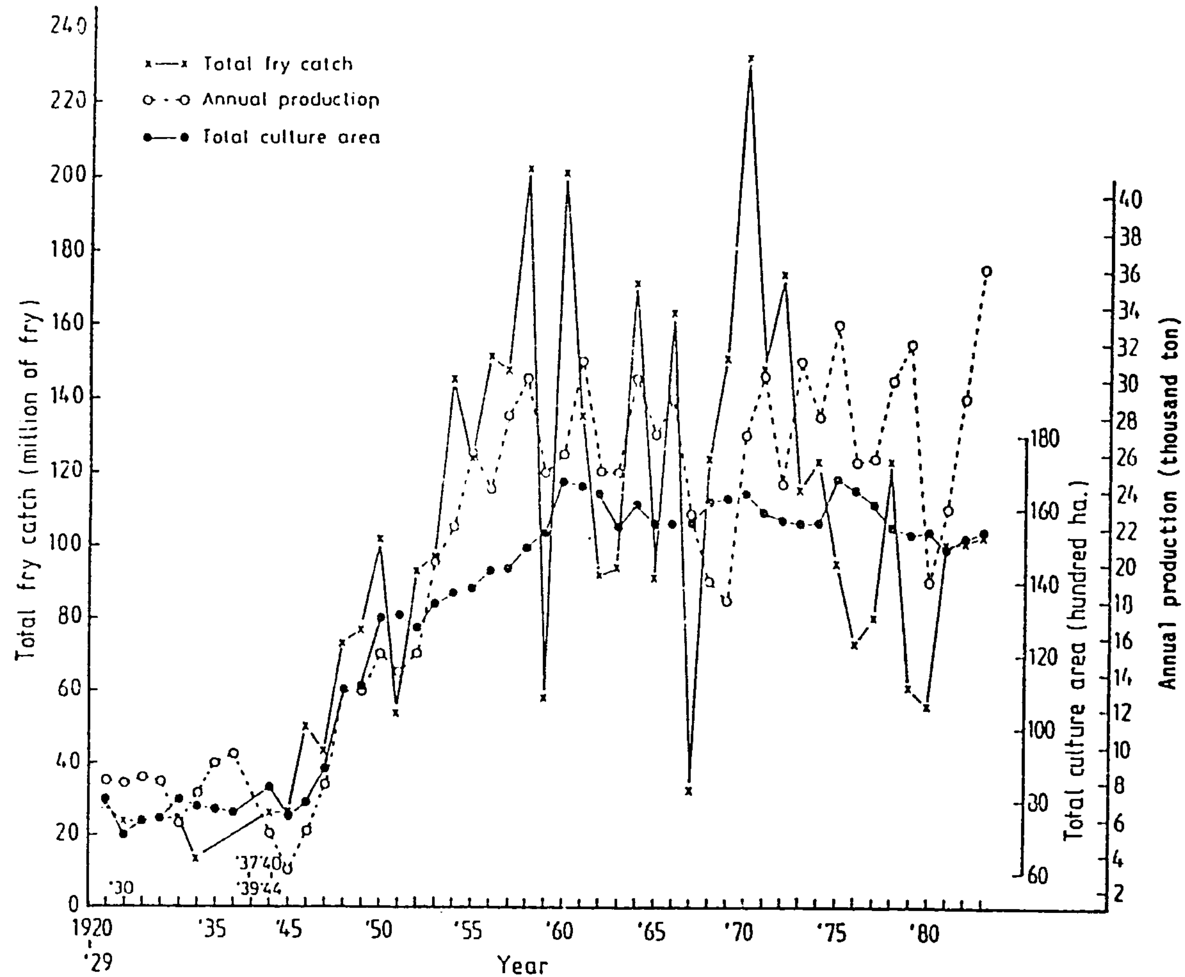


Fig. 1. Annual milkfish fry catch, production and culture area in Taiwan from 1920 to 1983.

is shown in Fig. 2 (Lee, 1981).

Methods of handling milkfish fry after collection are critical to their survival. In most areas, sorting and counting are done immediately after collection using a small, white plastic bowl. Unwanted species and dead fry are discarded. Reducing water salinity to 10-15 ppt to diminish osmotic stress on the fry can minimize mortalities. After collection, the fry are sent to fry gatherers or dealers who have storage facilities to accommodate them. In Taiwan, most of the fry are kept in 1.2 X 1.5 m^2 to 3 X 3 m^2 concrete tanks with a water depth of 15 cm. The stocking density is about 5,000/m^2 and the fry are fed wheat flour or egg yolk. The salinity of the water is maintained at around 12-20 ppt and the fry are then acclimated to the desired salinity. Finally, the fry dealers distribute their fry to rearing ponds for market-size milkfish (58%), nursery ponds for over-wintering fry (23%) and rearing ponds for baitfish (19%) (Lee, 1982). Although optimum salinity ranges for each growing stage are not yet confirmed, it has been found that for culturing fingerlings, lower salinities (2.7-8.6 ppt) are better than higher salinities (Hu and Liao, 1976). Salinities ranging from 12-30 ppt are suitable for growout (Chang, 1977).

Transportation of milkfish fry and fingerlings by the plastic bag method is common. Cylindrical plastic bags, 90 cm in circumference and 100 cm long, are filled with 10 liters of diluted seawater (about 7.5-10 ppt) and about 15 liters of oxygen. The stocking rates for each bag are 4,000-5,000 for fry, 1,000-2,000 for fish 3-5 cm total length (TL), 600-1,000 for fish 5-10 cm TL and 100-200 for fish 10-15 cm TL. When necessary, ice is placed outside the container to decrease the water temperature and metabolic rate of the fish. Mortality is less than 1% after two to three hours transportation.

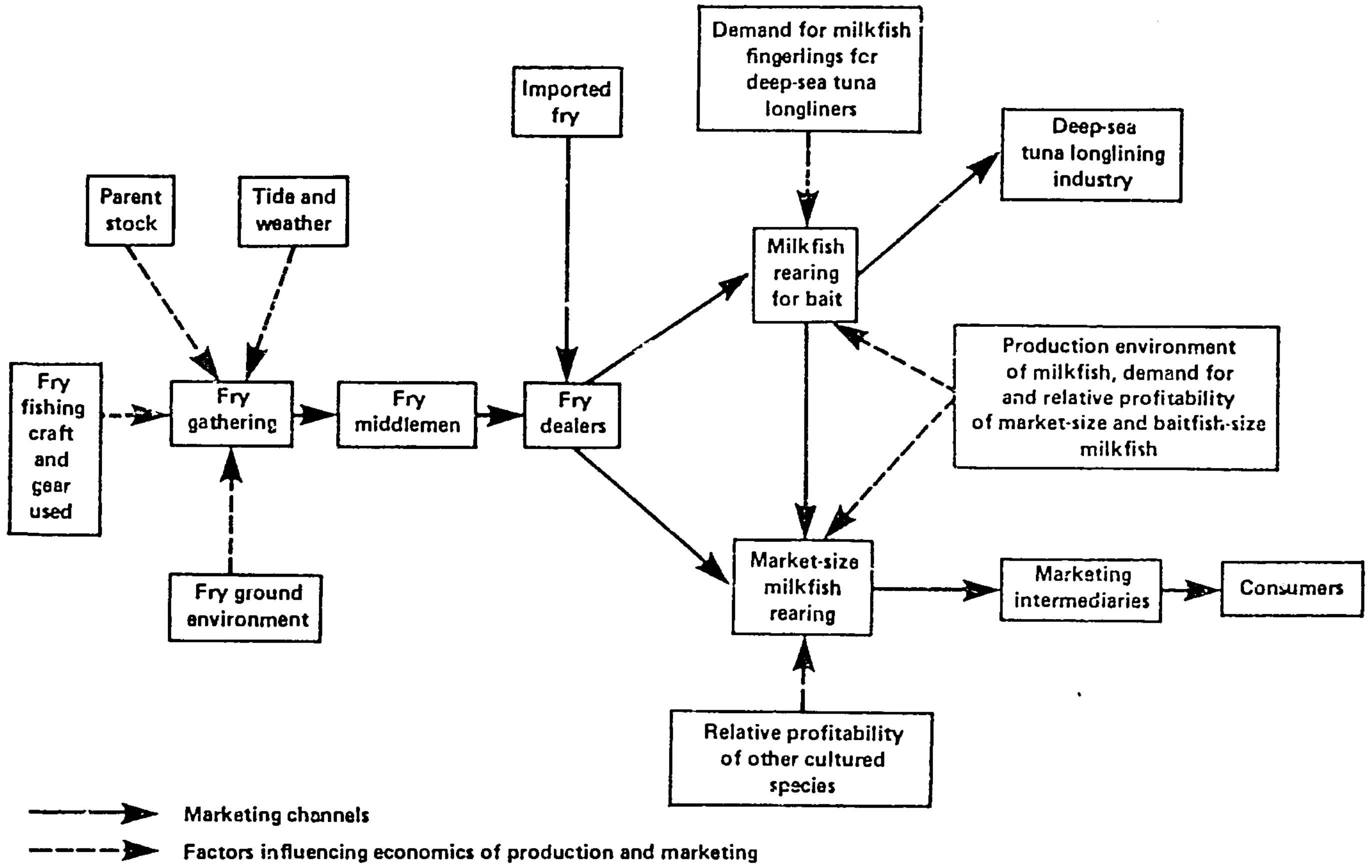


Fig. 2. The structure of the milkfish system in Taiwan (Lee, 1981).

8-3. FISHPOND DESIGN AND CONSTRUCTION

The size, location and system of milkfish ponds vary with local topography, climate and preference of the farmers. In Taiwan, the size of milkfish ponds ranges from 1-6 ha for either freshwater or brackishwater culture. In the Philippines and Indonesia, the pens or ponds may be as large as 100-500 hectares. The construction of the ponds is also influenced by local patterns of culture.

In Taiwan, for traditional brackishwater milkfish culture, tidal flats are ideal sites for constructing ponds; high tide can cover about 60 cm of pond depth and water can drain off at low tide. The optimum elevation for fishponds is from mean sea level to 0.45 m. Another consideration in site selection is the availability of enough underground or river water to regulate the salinity of rearing water and an adequate transportation system. The ideal soil bottom for milkfish ponds is silty loam, which retains water and provides conditions for the growth of benthic algae (Chen, 1971). The pond bottom soil, however, can be improved by fertilization.

The general layout and construction of shallow-water milkfish ponds in Taiwan consists of the following (Tang, 1962; Lin, 1968):

a. An outer dike used for protection from high waves (usually 3-5 m high, 1:2.5-3.0 slope, 3.7-5.0 m wide on top), inner dikes for water supply and drainage (1.7 m high, 1:1.5-2.0 slope, 1.5-3.7 m wide on top), small dikes for separating the different ponds (0.8-1.0 m high, 1:1.5 slope, 0.5-0.7 m wide on top) (Fig. 3a).

b. Water supply and drainage facilities that consist of a main canal (connecting to the sea, usually 12-15 m wide, depth depends on tidal conditions), subcanal (6m wide, 1.5 m deep), fish passageways, and water gates (Fig. 3b).

c. Nursery ponds, overwintering ponds and production ponds that are used for nursery, overwintering and growout of the milkfish. The production ponds are 3-5 ha in area and are usually longer in an east to west direction than north to south, with a 1-3:10,000 decline to the water gate. Nursery ponds are 2.5-3% of the area of production ponds and are located near overwintering ponds. Overwintering ponds are built in places where water exchange is convenient. These canal-shaped ponds are 1.5-2.0 m deep with wind breaks along the northern side.

For deepwater pond culture systems, the dikes are usually strengthened with bricks or cement to counter the increase in pond depth. Pumping devices for underground water are sometimes necessary for additional water supplies.

8-4. CULTURE METHODS AND MANAGEMENT

Milkfish monoculture can be practiced in freshwater, brackishwater or seawater ponds. Culture methods such as cage culture, pen culture, pond culture (including the "lab-lab" method or shallow-water pond culture system and the deepwater pond culture system), are used in Southeast Asian countries. Polyculture with other aquatic items is also practiced in some areas.

8-4.1. CAGE CULTURE SYSTEM

Cage culture of milkfish in the Philippines is conducted mainly for broodstock maintenance. Floating cages of different dimensions (3-10 m diameter X 1.5-3 m deep) are put in tidal coves and milkfish spawners are reared (Fig. 4) (Yu et al., 1979). If ecological factors are similar to the natural habitat, then the fish can mature sexually and spawn spontaneously (Lacanilao and Marte, 1980).

8-4.2. PEN CULTURE SYSTEM

Pen culture of the milkfish has been practiced for many years and has contributed greatly to total milkfish production in the Philippines. In Laguna de Bay, fishpens are constructed in various sizes and shapes: square, circular

Fig. 3a.

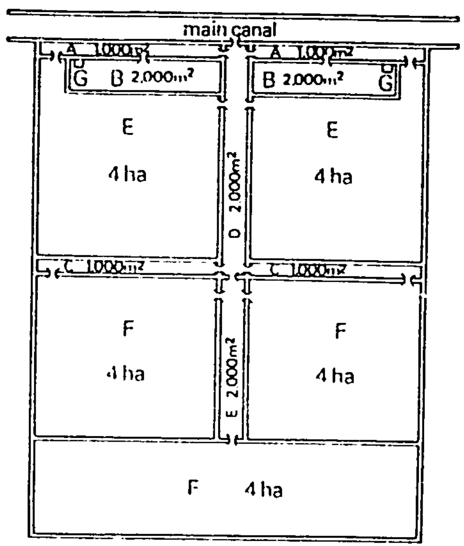


Fig. 3b.

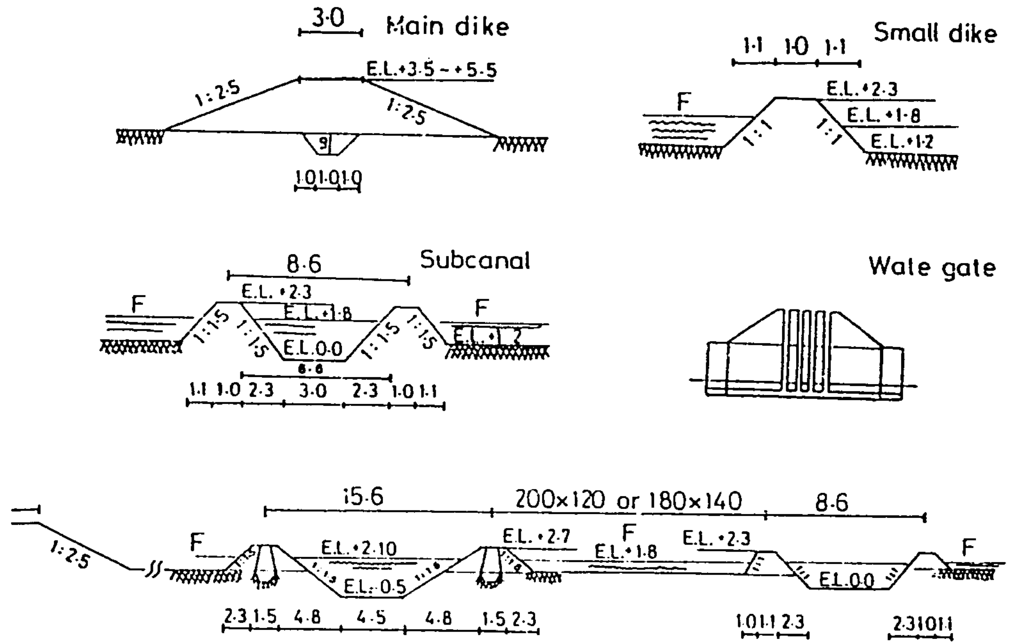


Fig. 3a. General layout of a 21.2 ha pond system. A = overwintering ponds and passageways, B = nursery ponds, C = passageways, D = subcanal, E = subcanal and passageways; F = production ponds, G = acclimation pools (After Lin, 1968).

Fig. 3b. Design and construction of dikes, canals, water gates and ponds.

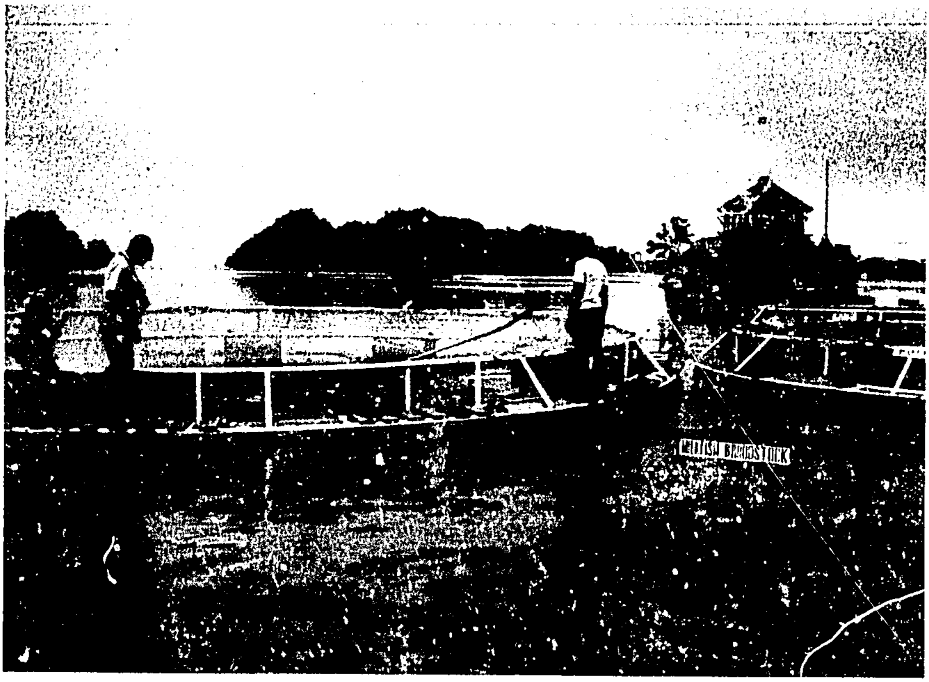


Fig. 4. Floating cage culture for milkfish spawners at Igang Substation, SEAFDEC, the Philippines.

Fig. 5. Pen culture in Laguna de Bay, the Philippines.

and rectangular (Fig. 5). They are stocked with milkfish fingerlings at densities 10-12 times higher than those in brackishwater ponds, and it has been observed that the growth rate of the fish is very high. The fish reach market size in five to six months, feeding only on the natural plankton in the lake water. In 1971, the culture of milkfish in pens was begun on a pilot scale of 38 ha in Lake Laguna, the largest lake in the Philippines. The fish pens were expanded to cover 4,800 ha by the end of 1973 (Delmendo and Gedney, 1976). In 1979, fish pens covered an area of about 7,000 ha and produced an average of 5,000 kg/ha/yr. Twelve years after the first fish pen was set up in the lake, about 34,000 ha of fish pens have been established (Baguilat, 1979; Pamplona and Mateo, 1985).

Based on previous experience with conditions of lake productivity, the stocking rate generally adopted for milkfish is about 30,000-40,000/ha in the rearing pen where they are grown to market size (Estrella, 1980; Baliao, 1984). Optimal stocking conditions occur from March to April when there is a relative abundance of fry and fingerlings. The plankton growth in the lake is relatively luxuriant during that period, and harvesting can be conducted before the onset of the typhoon month (Mane, 1975). The milkfish fingerlings usually stay in the prepared nursery compartment until they are large enough (about 12.5 cm) to be released into the rearing pens. In these pens, mortality may range from 20-40%. Partial or total harvesting is done from September on, depending upon market demand as well as the desired market size of the fish. The fish are caught by seine or gill nets, loaded into boats with ice and transported directly to market or processing plants.

Culturing milkfish in pens has the following distinct advantages:

1) Very high production in very short culture periods.

A potential annual yield of about 4,000-5,000 kg/ha, or over 10 times that of the open-water catch.

2) The spaces between fish pens serve as a refuge and breeding grounds to sustain natural fish production in the lake.

3) An abundance of natural food in the lake which makes supplemental feeding minimal

4) Growing fish in pens increases the income of the fish farmers and thus raises their living standard.

5) It can serve as a source of livelihood for the lakeshore inhabitants and also helps improve the economic condition of the towns bordering the lake.

There are some disadvantages and risks in operating milkfish pens, including the following:

1) The perennial threat of damage to infrastructure and escape of the stock arising from adverse weather conditions such as typhoons and floods.

2) Risk in the selection of site, construction and management of the fishpens.

3) Risk in acquiring construction materials and fingerlings for stocking.

4) Large accumulations of water hyacinth between fish pens and along navigation channels. This may cause breaks in the nets and framework collapse.

5) Risk of "mass fish kill" caused by "bad water" phenomenon in the lake. This may result from the intrusion of pollutants and sediments from the lake's tributaries and from decomposition of the thick algal population which then causes the localized depletion of oxygen in the lake water.

6) Risk of poaching.

8-4.3. SHALLOW-WATER POND CULTURE SYSTEM

The general practice of the shallow-water pond culture system is similar in Taiwan, the Philippines and Indonesia. This method applies techniques such as complete drying, tillage, fertilization of bottom soil to grow benthic algae as food for the milkfish, maintenance of water level (around 30-45 cm deep), mixed stocking and selective harvesting. Although Taiwan suffers a three-month cold winter period, annual production is higher (2,000-2,500 kg/ha/yr) than in both the Philippines and Indonesia (Huang, 1974).

In the Philippines, this culture system includes three culture methods (extensive farming system, mixed-size group culture and modular pond system). These culture methods determine the feeding scheme being adopted, the stocking density and the method of stock transfer in ponds. Except for the schedule of events, pond preparation for the three methods is similar to the traditional shallow-water pond culture method in Taiwan. The extensive system is considered the traditional method of milkfish farming. The fish stay in the same ponds from the time they are stocked to the time they are harvested and they feed only on lab-lab (benthic algal mixture). Harvesting of the fish is done at one time. The mixed-size group culture is a modification of the traditional method with multi-size stocking and selective harvesting. The modular pond method is a more complicated but highly productive type of culture method. It has been adopted by Philippine farmers in recent years. Fish rearing comprises three production stages. As the fish grow, they are moved from a smaller to a larger compartment. The culture period in a pond lasts from 30 to 45 days. Once vacated, each pond is immediately prepared to receive incoming stock. The areas of the various ponds are in a proportion of 1:2:4 for nursery, transition and rearing ponds, respectively. The modular system has many variations. It is a continuous program of pond preparation, stocking, transfer and harvest, allowing as many as six crops per year (Pamplona and Mateo, 1985).

The management of shallow-water milkfish culture in

Taiwan involves several steps: a) preparation of the ponds (November to March); b) stocking and stock manipulation (April to August); c) maintenance of water conditions and growth of food organisms (April to October); d) control of pests, predators and diseases; e) selective harvesting and marketing (June to October); and f) overwintering (November to March). The complete list of procedures and schedules is in the flow chart (Fig. 6).

1) Pond preparation (Fig. 7)

In Taiwan, preparation of the milkfish ponds is conducted from November to March. After the milkfish are harvested or removed to overwintering ponds, the production ponds are drained and sun-dried until the bottom soil cracks. Repair of the dikes, water gates and water supply canals, as well as tilling and leveling of the bottom to regenerate the productivity of the ponds, is also done during this period. The ponds are then refilled with 5-20 cm of water and allowed to evaporate until completely dry. The process is repeated two or three times. Spreading fertilizer over the pond bottom is indispensable for growing a benthic algal mat for milkfish grazing. Primarily, organic fertilizers are used at 500-1,000 kg/ha for chicken manure or 300-500 kg/ha for rice bran. Fresh seawater is added to a depth of 10-15 cm. After several weeks, the organic nutrients accumulate at the pond's bottom and benthic algae grows to a thickness of about 1-3 mm. Liming is sometimes necessary when the ponds become acidic; this also helps to kill the pests in the ponds. Repeated treatment of flooding, fertilizing and evaporating the ponds is called the "wintering treatment". Two to three weeks before the stocking of fingerlings in March and early April, tea seed cake (containing 7-8% saponin) or tobacco waste (containing 3% nicotine) is added to the ponds at a rate of 150-200 kg/ha each to kill pests and predators (Chang et al., 1977). Water is then reintroduced to the ponds to a

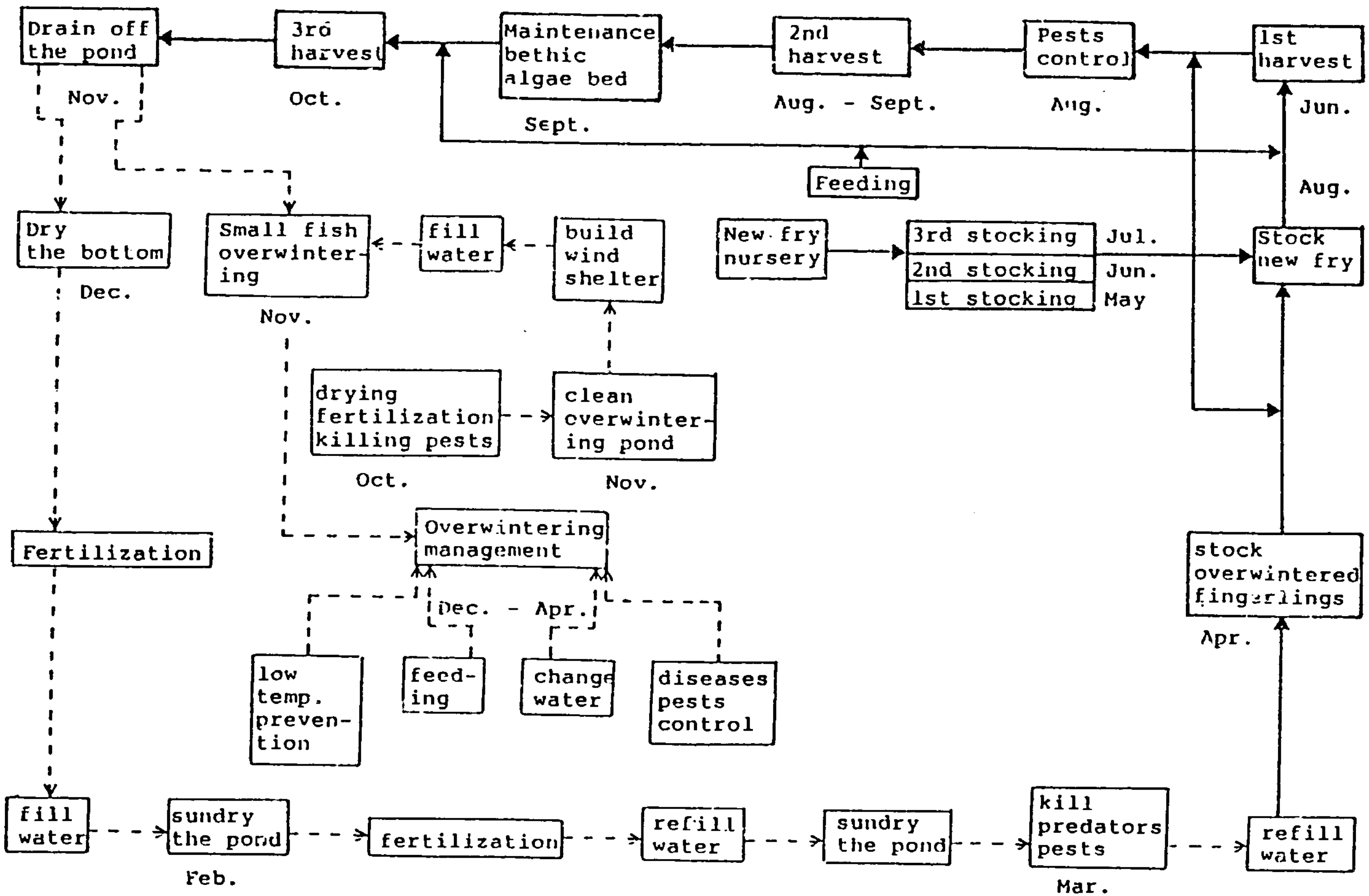


Fig. 6. Flow chart for traditional shallow-water milkfish culture system. Dashed line shows preparation stage and solid line shows rearing and production

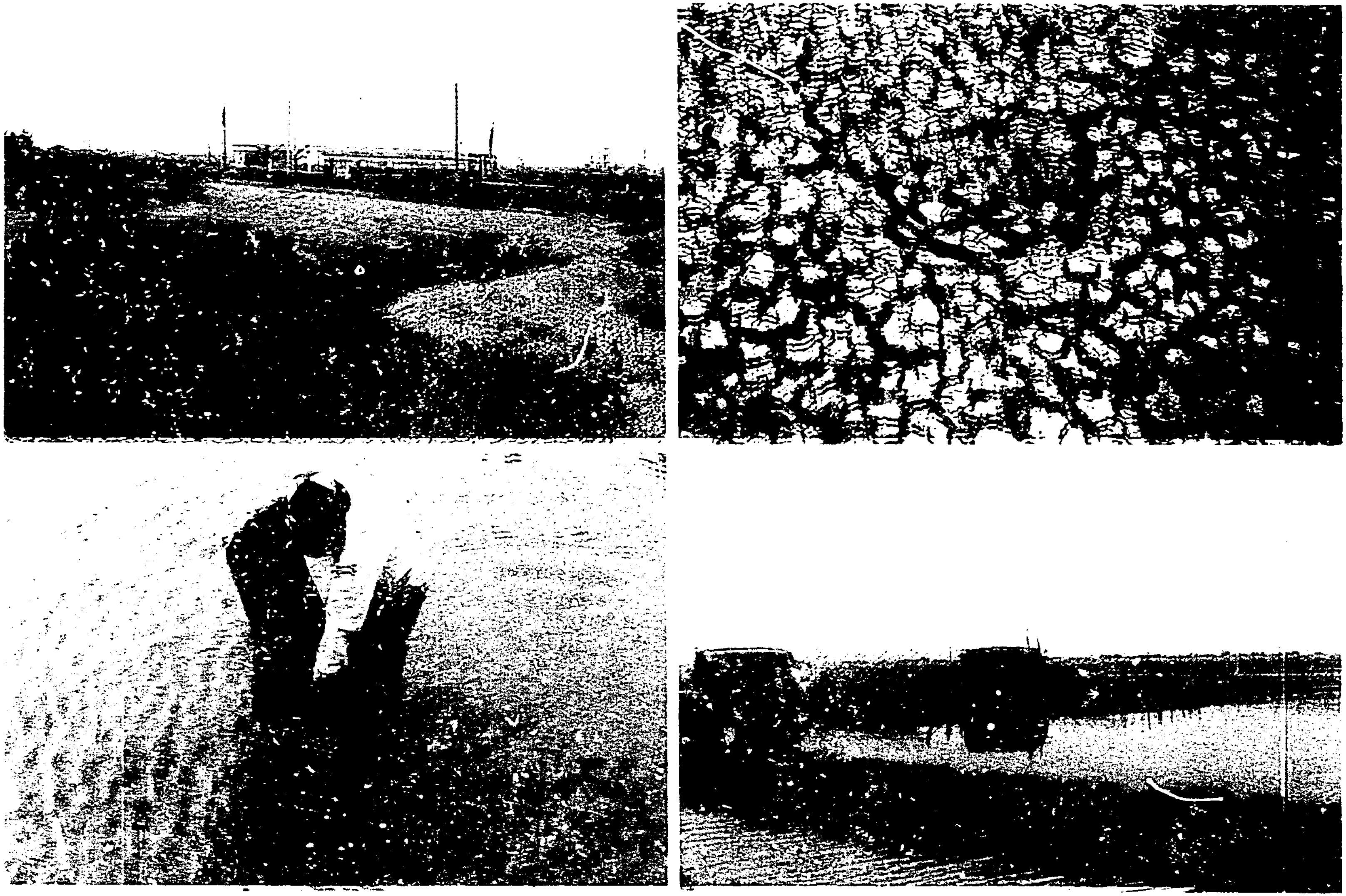


Fig. 7. Traditional shallow-water milkfish pond culture in Taiwan, with emphasis on pond preparation, stocking and management.

depth of about 30-45 cm. At this time, the ponds are ready for stocking.

2) Stocking and stock manipulation (Fig 7.)

Multiple-size stocking is adopted in Taiwan during the stocking season, i.e., April to August. Repeated stocking is done with fingerlings and fry of different sizes. Large overwintered fingerlings (50-100 g/fish), medium overwintered fingerlings (10-50 g/fish), small overwintered fingerlings (3.5-10 g/fish), and new fry caught in fry season are stocked at a rate of 2,000-2,500, 2,000-2,500, 1,500-2,000, and 6,000-7,000 fish/ha, respectively. Thus, the general stocking pattern practiced in Taiwan is 4,000 to 5,000 over-wintered fingerlings of 5 to 100 g/ha in April plus 5,000 to 8,000 new fry to stock during the period from May to August (Lin, 1968). Presently, stocking rates of some milkfish ponds are kept low at 4,000 fingerlings/ha to promote faster growth of the fish. Fish are first stocked in the nursery pond and then in production ponds. Before stocking the fish into production ponds, fry and fingerlings are acclimated to the salinity required in the nursery ponds. This increases survival rate after stocking.

3) Maintenance of water conditions and food organisms production

The routine work which takes place after the fish are stocked includes maintaining good water quality and growing enough food organisms for the milkfish. The production of adequate natural food in milkfish culture ponds is crucial to successful farming. It is reported that genera of blue-green algae (Oscillatoria, Lyngbya, Phormidium, Spirulina, Micro-coccus, etc.) and diatoms (Navicula, Pleurosigma, Mastogloia, Stauroneis, Amphora, Nitzschia, etc.) constitute the major part of the food content in pond-reared milkfish and that they are the most abundant algae in a benthic algal mat (Lin, 1967). In general, the ponds are fertilized to grow these natural foods for the milkfish.

It is also very important to maintain water level and salinity in the ponds during the culturing period. Evaporation and heavy rainfall change the salinity of water, and may damage the benthic algal mat and cause blooming of unsuitable planktons. In a well-conditioned milkfish pond, the water is always clear with benthic algae in rapid growth. Blooms of other microplanktons, such as protozoans, flagellates, phytoflagellates and rotiferans turn the clear water a yellowish or brownish color. Consequently, the low level of dissolved oxygen in the water may cause mass mortality of the milkfish. Fresh seawater needs to be filled and changed continuously. Fertilization and supplemental feeding with rice bran or peanut cake, at a rate of 30 kg/ha, may sustain the growth of the fish.

4) Control of pests, predators and diseases

The most common predators, competitors and pests found in milkfish ponds are Elops saurus and Megalops cyprinoides (predators); Chironomid larvae of Tendipes (Chironomus) longilobus (competitor for food); Batillaria sp., Corithida sp., Nereis gladicincta and Oreochromis mossambicus (food competitors and destroyers of algal mat); Apolechins latipes, Gambusia affinis, and Neoceridina denticulata (nuisance fish and shrimp). Unwanted organisms should be killed before stocking of fingerlings, and screen nets should be placed in water gates to prevent pests or predators from entering the ponds. For killing snails, a 24-hour treatment of 0.35 ppm Bayluscide at a water temperature of 30°C or 72 hours with 1 ppm Brestan 60 are effective. Nereis sp. can be killed with 3 ppm saponin or nicotine. Pesticides, such as 34% Sumithion, have been used to eliminate Chironomid larvae. The effective dosage is 0.13 ppm for a 24-hour treatment (Lin, 1981c). Also, biological control with hydrophilid beetle larvae, Berosus (Enoplurus) fairmaiyei (Lin, 1968), is also effective.

In conclusion, the necessary conditions for growing and maintaining benthic algal mat for milkfish stocking are:

a. Fertile clay, clay loam soil;
b. Pond water depth of about 10-15 cm during the development of benthic algae and later an increase to 30-40 cm or more during the growing period of milkfish;
c. Salinity ranging from 10-40 ppt;
d. Avoiding disturbance of the pond;
e. Freshening the water to enhance further growth of benthic algae;
f. Control of pests and predators that feed on or destroy natural food in the ponds.

5) Selective harvesting and marketing

After being reared to baitfish size (80-120 g) or food fish size (200-500 g), selective harvesting of milkfish with various mesh-size seine nets is carried out, depending on the demands of the consumer market or bait needs of the tuna longline fishery (Fig. 8). Harvesting usually begins in May and continues through November. Fish farmers usually empty the digestive tracts of the milkfish by exciting them before harvesting. The wholesale price of milkfish is about US$2/kg (Taiwan Fisheries Bureau, 1983).

6) Overwintering

Tainan and Kaohsiung counties of Taiwan have a three-month winter period when the water temperature sometimes drops to 10°C. It is necessary to construct overwintering ponds to protect the fish from the chilly northerly wind and any other sudden change of weather. The overwinterin[illegible]nd, usually long and narrow, lies east to west and is about 5 m X 100 to 200 m and 1.5 to 2 m in depth. The pond is connected to a nursery pond by a narrow opening. A wind break is made along the north side with materials such as straw, couch

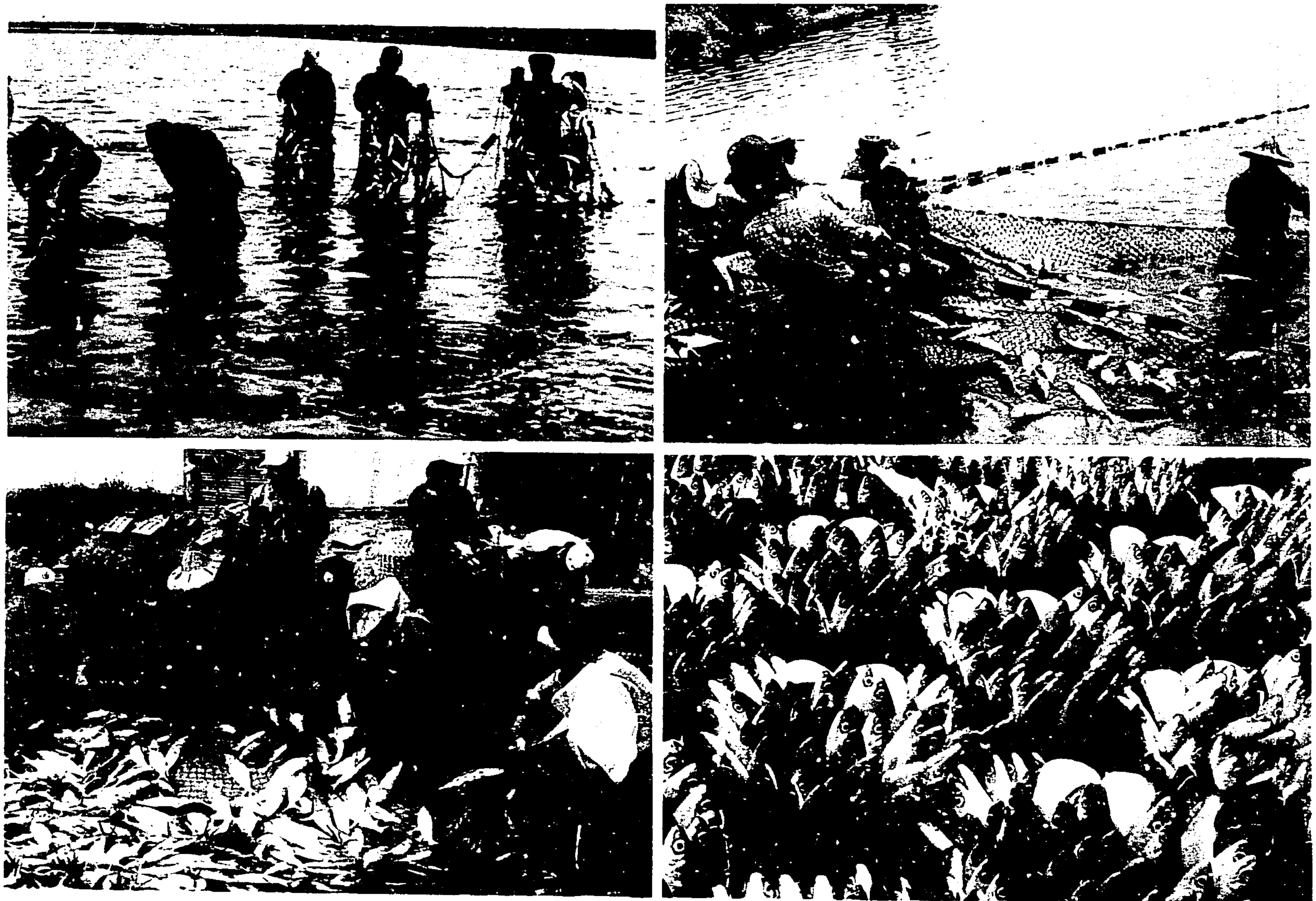


Fig. 8. Harvesting of the market-size milkfish by gill net.

glass, canvas or polyethylene (PE) cloth. Additional PE plastic often covers the wintering canal to maintain a higher water temperature for the overwintering ponds (Ting, 1978) (Fig. 9). Each year before November, overwintering ponds are prepared to accommodate the fish that are smaller than market size. Stocking density depends on size of fish, depth of overwintering canal and other environmental factors. The optimum stocking density is less than 1.3 kg/m^3. The fish are fed with rice bran in the nearby nursery pond when the weather turns mild. Mortality varies from 0.6 to 87% because of the problems associated with low temperature, unsuitable stocking density, aging of overwintering ponds and diseases. Studies on improving construction, water supply systems, stocking rates and disease control of overwintering ponds have been done by many researchers (Ting, 1978; Lin et al., 1981; Lin, 1982; Ting et al., 1984) but effective measures have yet to be developed.

8-4.4. DEEPWATER POND CULTURE SYSTEM

In the mid-1970s, the deepwater pond culture system was developed in response to 1) limited land, 2) limited manpower supply in rural areas, 3) relatively low market price for other freshwater fishes when compared with milkfish, 4) availability of formulated feeds, and 5) lack of necessity for overwintering ponds because of stable water temperatures. The traditional, shallow-water method, practiced especially where the salinity is low, is changed to the deepwater practice by increasing the depth of ponds to 3-10 m and subdividing large ponds into smaller ones (about 1 ha each). With this method, stocking density is increased three to five times that of the traditional method. In general, about 25,000 or more fish are stocked per ha. Aerators and automatic feeders are equipped with devices to compensate for dissolved oxygen depleted by the high stocking density and insufficient supply of benthic algae caused by deepening the

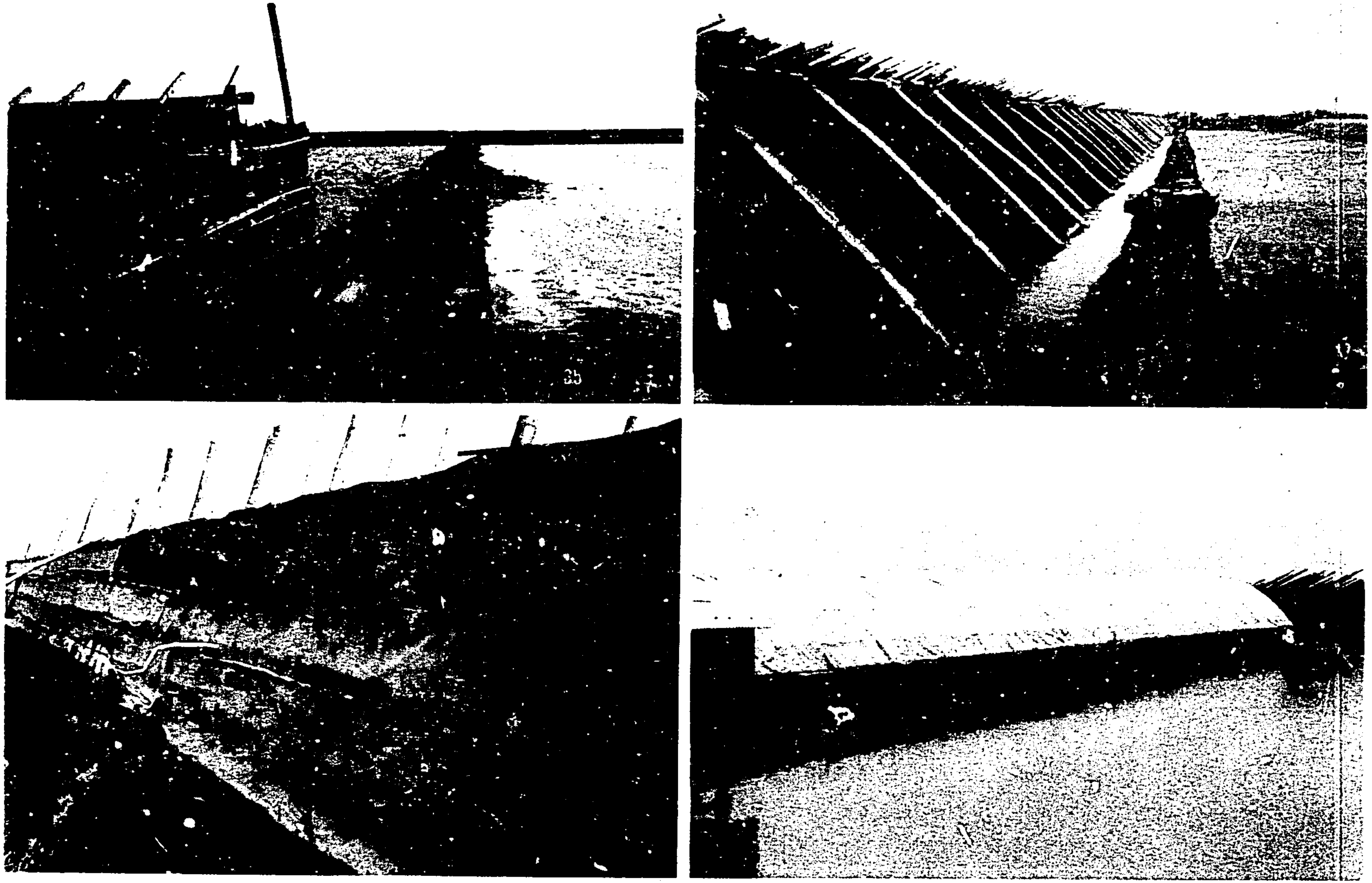


Fig. 9. Overwintering canal using different construction materials for wind breaks.

pond water (Fig. 10 a & b). An annual production of 8,000-10,000 kg/ha has been achieved and, in some cases, production reaches as high as 11,760 kg/ha (Chen, 1981; Huang, 1981; Lin, 1981a, b, c, d).

The general practices of the deepwater pond culture system are as follows:

1) Pond preparation

This process is usually similar to that of the traditional method. Complete drying, tillage and liming of the bottom soil are necessary while fertilization is provided as required.

2) Stocking

This is performed after April. Milkfish fingerlings of 1.5 cm TL are first stocked at a rate of about 12,000/ha. The second stocking of 13,000/ha is done after selective harvesting of market-size fish.

3) Management

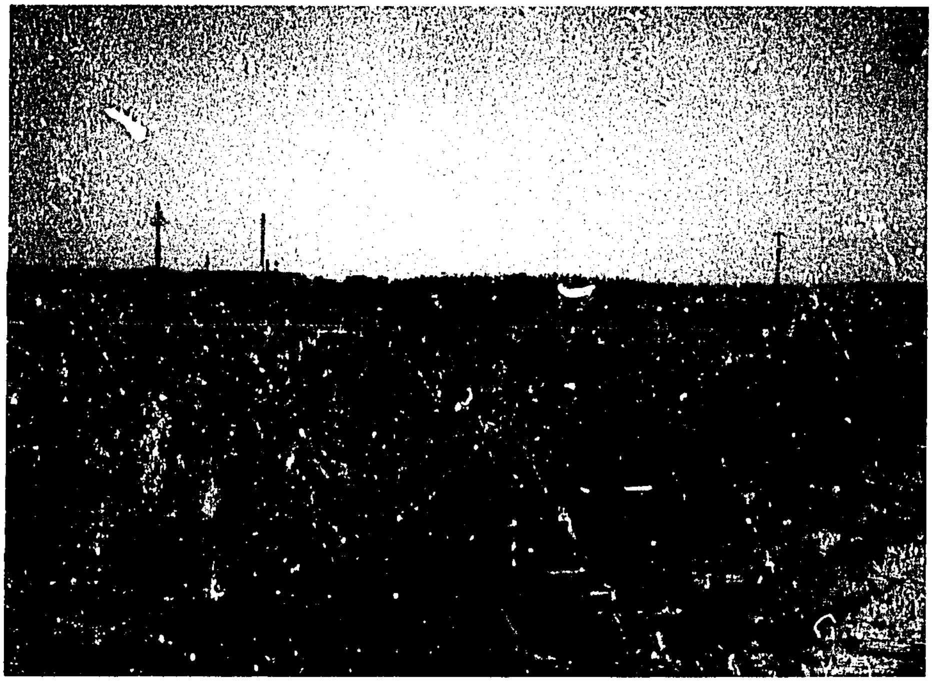
An automatic feeder dispensing artificial feeds and two paddlewheel aerators are installed per hectare pond. Training of the milkfish fingerlings to the feed is necessary for two or three days. Feeding is carried out twice a day after the fourth day. Pelleted milkfish feeds (23-27% crude protein) are given according to body weight increase of the fish and an expected feed conversion rate of 1.3. Pond water is maintained at 2-3 m in depth. Maintaining good water quality and control of plankton biomass and feeding rates are also needed.

4) Harvesting

Selective harvesting using gill nets is executed after July to decrease stocking density and obtain maximum growth of the fish. Milkfish with body weights over 500 g are harvested.

5) Overwintering

After the November harvest, milkfish smaller than



b

Fig. 10 a & b. Deep-water milkfish ponds with paddle-wheel aerators and automatic feeders.

market-size are removed to overwintering ponds. Disease prevention and control is the most important activity at this time. In March to May of the following year, the fish grow to market size and are harvested. It is the most profitable harvesting period because market prices are highest in this period.

Major constraints to the deepwater pond culture method are the availability of fry and disease incidence caused by high stocking density, unsuitable feeding, and overwintering. This practice also involves a higher risk than the traditional method.

8-4.5. POLYCULTURE SYSTEM

Polyculture of milkfish with tilapia hybrid, mullet and other Cyprinidae fish has been carried out for centuries in most freshwater ponds. In response to the pressure of declining profits within the milkfish industry, brackishwater ponds are increasingly being used for polyculture with other species such as shrimps (*Penaeus monodon* and *Metapenaeus monoceros*), crab (*Scylla serrata*), other economically valuable fish (*Lates calcarifer*), and algae (*Gracillaria* sp).

The ratio of milkfish to other aquatic organisms depends on the farmers' manipulations and preferences, as well as on market demand. Experiments on polyculture of milkfish with *P. monodon* indicate that milkfish grow better at a milkfish to shrimp stocking ratio of 1:25.

8-4.6. INTEGRATED CULTURE SYSTEM

Integrating freshwater culture of milkfish and tilapia with pig rearing has been successful with daily application of pig manure or wastes and appropriate fish stocking density (Tamse, 1983). Experiments on integrated farming of milkfish, *P. indicus*, and poultry in brackishwater ponds have also been undertaken by Apud and Pudadera (1983).

8-5. HARVESTING, MARKETING AND PROCESSING

As mentioned earlier, timing for harvesting milkfish depends on the demands of the food fish market and tuna longline fishery. In Taiwan, the newly-stocked fry or fingerlings of 5 cm TL attain baitfish size (80-120 g) and food fish size (200-500 g) after two to three months and six to eight months of rearing, respectively. The market channel that links producers and consumers is relatively short: producers--> dealers--> city fish market--> retailers--> consumers. In general, the net return rates for baitfish rearing and food fish rearing are 29.8% and 9.3%, respectively (Lee, 1981).

Milkfish is consumed primarily fresh or frozen in Taiwan in accordance with consumer preference. Thus far, the percentage of total milkfish production canned or smoked is relatively low. This situation is similar in the Philippines and Indonesia.

8-6. STATUS AND PROSPECTS

The three major countries culturing milkfish on an extensive scale are Taiwan, the Philippines, and Indonesia. Traditionally, milkfish farming is based on a natural ecosystem equilibrium where the growth of benthic algae provides food for the milkfish. It is an economical way to produce animal protein. With the expansion of the milkfish industry, a more intensive culture style (deepwater pond culture) has been developed. More operating input is demanded by this method. With milkfish culture in Taiwan as an example, a comparison of shallow-water and deepwater pond culture practices is shown in Table 1. Table 2 summarizes and compares the advantages and disadvantages of the two culture styles (Liao, 1985). In Taiwan, increases in production resulting from the improvement of culture techniques and the alteration of the consumption pattern have caused the retail price of market-size milkfish to drop to less than US$2/kg in recent

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Table 1. Comparison of the present status between shallow-water and deep-water pond culture systems conducted in Taiwan.

Items	Shallow-water	Deep-water
Pond area (ha)	1-10	1-6
Water depth (m)	0.35-0.40	2-3
Fertilization	organic fertilizer added	not necessary
Aeration	none	Paddlewheel - 2/ha
Feeding	natural food supplemented with rice bran 30 kg/ha when necessary	artificial feed (26% crude protein) 2,160 kg/ha
Overwintering	facilities needed	not necessary
Stocking rate (fish/ha)		18,000-50,000
a. overwintered fingerling	3,500-4,500	
b. new fry	6,000-7,000	
Production (kg/ha)	2,000-2,500	8,000-12,000

Sources: Chen, 1976 & 1981; Huang, 1974 & 1981.

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years. Facing a decline in land availability and profitability, many milkfish farmers have begun to culture other high-value species (shrimps and crabs, especially P. monodon and S. serrata) with milkfish, or totally shift to culturing other species. The longline fishery demand for baitfish also encourages some milkfish farmers to specialize in fingerling production for baitfish. Thus, in recent years, milkfish farming has become less attractive than shrimp farming.

In the Philippines, milkfish culture areas and production steadily increased from 1955 to 1982. Of the present 208,120 ha of fish ponds, 195,830 ha are brackishwater ponds, and of these, 91% are milkfish ponds. Furthermore, 62,000 ha

Table 2. The advantages and disadvantages of traditional and modern styles of milkfish culture in Taiwan (Liao, 1985).

TRADITIONAL STYLE (shallow-water pond culture system; fertilizing culture method)	MODERN STYLE (deepwater pond culture system; feeding culture method)
Advantages:	Advantages:
* A satisfactory culture method by utilizing a balanced natural ecosystem	* Less attention to water preparation and maintenance
* Feed-saving and energy-saving	* Much higher stocking density (5 times or over)
* Healthy fish, almost no disease	* Much higher yield (4 times or over)
Disadvantages:	* Fast growth
* Maintenance of water quality is very sensitive and experience-dependent	* No need for additional over-wintering facilities. Continuous production in winter months when high prices can be guaranteed
* Maximum yield is limited to a relatively low level	Disadvantages:
* Better growth rate can only be obtained by lowering the already low density	* Comparatively expensive facilities
* Overwintering facility is needed	* High investment risk

of mangrove marsh are available for further fishpond development (Samson, 1984). In Indonesia, milkfish farming covers an area of 182,000 ha and another 6 million ha of tidal land are reported to be suitable for brackishwater milkfish culture (Chong et al., 1984).

8-7. PROBLEMS AND RECOMMENDATIONS

The existing problems in milkfish farming are:

1) Seed supply

In Taiwan, the major constraint of intensified milkfish culture is inadequate supplies of fry when needed for stocking. Currently, some techniques for artificial propagation have been established but massive production of fry is still unattainable (Liao and Chen, 1984; Lin, 1984). Fry supply still depends mainly on collection from the wild or import from other countries. The fluctuation of the fry supply has hampered rational planning for further investment and development. In the Philippines and Indonesia, the annual catch is adequate to meet the demand for stocking. Distribution is the major problem in these countries.

2) Overwintering

During the cold winter period in Taiwan, high mortality of milkfish sometimes occurs because of low temperatures, oxygen depletion and outbreaks of disease. This causes a severe shortage of overwintered fingerlings for stocking (see Chapter 9).

3) Lack of information on ecology and physiology of milkfish fry, fingerlings and adults

Fry collecting methods and culture techniques can be improved based on the knowledge of milkfish biology, habitat and feeding behavior, etc.

4) Pond cultivation and management

Methods of pond preparation, fertilization, water quality control, optimum stocking density and pond management should be revised and established to attain maximum production.

5) Nutrition and diet development

Artificial feeds for milkfish have been developed with crude protein values of about 23-29%. In Taiwan, there are 12 companies producing nearly 30,000 kg of feed a year with a price of about US$0.35-0.45/kg. Study of the nutritional requirements of milkfish is essential so that nutritious feeds can be developed.

6) Diseases

Identification of milkfish diseases in rearing and overwintering periods is also urgently needed.

7) Coordination of production and marketing

Overproduction and poor marketing may cause a decrease in consumption of the fish. These two factors may result in declining profit for the milkfish farmers. A policy to regulate production and marketing by controlling stocking and harvesting is necessary in order to achieve maximum profits.

8) Processing

The boniness of the milkfish affects the preferences and buying habits of the consumers. The further processing of milkfish into a boneless canned product may open a wider domestic and foreign market, which may stimulate consumption.

Specific recommendations for future work are:

1) More effort should be concentrated on mass seed production, especially hatchery-produced fry. The further development of environmental control and hormone manipulation of the spawners, as well as the improvement of larval-rearing techniques, will solve the problem of inadequate fry supply in the near future.

2) Refinement and standardization of the existing fry collection, handling and culture techniques, which were established by trial and error, are required to increase production and reduce labor.

3) Genetic studies to improve the quality of the milkfish should be undertaken.

4) Socio-economic evaluation should be carried out to document the needs of the milkfish industry.

5) Providing guidance in shallow-water milkfish culture to developing countries other than Southeast Asia may result in the best use of milkfish resources. Furthermore, the supply of animal protein may also be increased.

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REFERENCES

Apud, F.D. and B.J. Pudadera, Jr. 1983. Integrated farming of Penaeus indicus, Chanos chanos, and poultry in brackishwater ponds. Presented at 2nd Int. Milkfish Aquaculture Conf., Iloilo, Philippines, Oct. 4-8, 1983.

Baguilat, T. 1979. The fishpen industry of the Philippines: An overview. Int. Workshop on Pen and Cage Culture of Fish. SEAFDEC-IDRC Workshop, Tigbauan, Iloilo, Philippines, Feb. 11-22, 1979.

Baliao, D.D. 1984. Milkfish nursery pond and pen culture in the Indo-Pacific region. Proc. 2nd Int. Milkfish Aquaculture Conf., Iloilo, Philippines, Feb. 11-22, 1979. pp. 97-106.

Chang, M.H. 1977. Milkfish culture. Manual of Aquaculture No. 63, Taiwan Fish. Res. Institute. p. 18 (in Chinese).

Chang, M.H., S.S. Chen, K.Y. Lin and S.C. Chen. 1977. Experiment on application of tobacco waste as fertilizer and pesticide in milkfish pond. Bull. Taiwan Fish. Res. Inst. 29: 39-46 (in Chinese with English abstract).

Chen, S.H. 1971. Study on the soil investigation of the milkfish pond in Taiwan. Bull. Taiwan. Fish. Res. Inst. 19: 41-45 (in Chinese).

Chen, T.P. 1976. Aquaculture practices in Taiwan. Fishing New Books, Ltd., Survey, England. p. 161.

Chen, T.P. 1981. Taiwan farmers go deep for milkfish. Fish Farming International 10: 5.

Chong, K.-C., A. Poernomo and F. Kasryno. 1984. Economic and technological aspects of the Indonesian milkfish industry. Proc. 2nd Int. Milkfish Aquaculture Conf., Iloilo, Philippines, Feb. 11-22, 1979. pp. 199-213.

Delmendo, M.N. and R.H. Gedney. 1976. Laguna de Bay fish pen aquaculture development - Philippines. J. World Maricul. Soc. 7: 257-265.

Estrella, J.J., Jr. 1980. Milkfish culture in pens in Laguna de Bay. Presented at the 3rd Aquabusiness Project Development and Management (APDEM) Seminar at Tigbauan, Iloilo, Philippines, Nov. 28, 1980. pp. 12.

Hu, F. and I.C. Liao. 1976. Effect of salinity on growth of young milkfish, Chanos chanos. Proc. Int. Milkfish Workshop Conf., Iloilo, Phillipines, May 19-22, 1976. Working Paper 2: 33-42.

Huang, T.L. 1974. Aquaculture in Taiwan: milkfish culture. Bank of Taiwan Quarterly Journal 25(1): 184-205 (in Chinese).

Huang, T.L. 1981. An investigation report of deepwater milkfish culture. China Fish. Monthly 338: 12-15 (in Chinese).

Lacanilao, F.L. and C.L. Marte. 1980. Sexual maturation of milkfish in floating cages. Asian Aquaculture 3: 4-6.

Lee, C.S. 1981. Production and marketing of milkfish in Taiwan: an economic analysis. ICLARM Tech. Rep. 6, p. 41.

Lee, C.S. 1982. Economics of Taiwan milkfish system. Aquaculture Economics Research in Asia, IDRC-193e, Ottawa, Canada. pp. 45-57.

Liao, I.C. 1971. Note on some adult milkfish from the coast of southern Taiwan. Aquaculture 1(3): 1-10 (in Chinese with English abstract).

Liao, I.C. 1985. Milkfish culture in Taiwan. In: C.S. Lee and I.C. Liao (Eds.) Reproduction and Culture of Milkfish. Oceanic Institute, Hawaii and Tungkang Marine Laboratory, Taiwan. pp. 164-184.

Liao, I.C. and T.I. Chen. 1984. Gonadal development and induced breeding of captive milkfish in Taiwan. In: J.V. Juario, R.P. Ferraris, L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc., Manila, Philippines. pp. 41-51.

Lin, C.L. 1982. Disease and its control of the milkfish during overwintering period. Fisherman Magazine 5(6): 42-44 (in Chinese).

Lin, L.T. 1984. Studies on the induced breeding of milkfish (Chanos chanos Forsskal) reared in ponds. China Fisheries Monthly 387: 3-29 (in Chinese with English abstract).

Lin, M.N. 1981a. Theory and practice of deepwater milkfish culture (I). Fisherman Magazine 4(5): 35-37 (in Chinese).

Lin, M.N. 1981b. Theory and practice of deepwater milkfish culture (II). Fisherman Magazine 4(6): 46-48 (in Chinese).

Lin, M.N. 1981c. Theory and practice of deepwater milkfish culture (III). Fisherman Magazine 4(7): 42-44 (in Chinese).

Lin, M.N. 1981d. Theory and practice of deepwater milkfish culture (IV). Fisherman Magazine 4(8): 39-41 (in Chinese).

Lin, M.N., B.S. Tseng, K.Y. Lin and Y.Y. Ting. 1981. Some improvements of milkfish, C. chanos, overwintering farming. Bull. Taiwan Fish. Res. Inst. 33: 695-701 (in Chinese).

Lin, S.W. 1967. Feeds and feeding of warm-water fishes in Asia and the Far East. Proc. FAO World Symposium on Warm-water Pond Fish Culture. FAO Fish Rep. 44(3): 291-309.

Lin, S.Y. 1968. Milkfish farming in Taiwan. Fish Culture Report No. 3, Taiwan Fish. Res. Inst. p. 63.

Mane, A.M. 1975. Some observations and experiences in pen culture for bangos in Laguna de Bay. Proc. Nat'l Bangos Symposium. Punta Baluarte, Philippines, July 25-28, 1975. pp. 112-124.

Pamplona, S.D. and R.T. Mateo. 1985. Milkfish farming in the Philippines. In: C.S. Lee and I.C. Liao (Eds.) Reproduction and Culture of Milkfish. Oceanic Institute, Hawaii and Tungkang Marine Laboratory, Taiwan. pp. 141-163.

Samson, E. 1984. The milkfish industry in the Philippines. Proc. 2nd Int. Milkfish Aquaculture Conf., Iloilo, Philippines, Oct. 4-8, 1983. pp. 216-228.

Senta, T. and A. Hirai. 1981. Seasonal occurrence of milkfish fry at Tanegashima and Takushima in southern Japan. Japan. J. Ichthy. 28(1): 45-51.

Schuster, W.H. 1952. Fish culture in brackishwater ponds in Java. Spec. Publ. Indo-Pacif. Fish Coun. (1). 140 pp.

Taiwan Fisheries Bureau. 1983. Fisheries Year Book, Taiwan area of 1982. Taiwan Fisheries Bureau, Dept. of Agriculture and Forestry, Provincial Gov. of Taiwan.

Tamse, A.F. 1983. The regular application of fresh pig manure for milkfish culture in brackishwater ponds. M.S. thesis, College of Fisheries, Univ. of the Phil. Visayas, Iloilo City. p. 75.

Tang, Y.A. 1962. Design and construction of milkfish ponds. Manual of Aquaculture No. 20, Taiwan Fish. Res. Inst. p. 16 (in Chinese).

Ting, Y.Y. 1978. The studies on milkfish overwintering. Bull. Taiwan Fish. Res. Inst. 30: 479-486 (in Chinese).

Ting, Y.Y., M.N. Lin and K.Y. Lin. 1984. Comparative experiments on the different kinds of materials for building the wind shelter of milkfish overwintering pond. Bull. Taiwan Fish. Res. Inst. 37: 117-127 (in Chinese with English abstract).

Yu, O.K., A.T. Vizcarra and H.S. Sitoy. 1979. Development of circular floating cages for milkfish broodstock at the SEAFDEC Aquaculture Department. Proc. Int. Workshop on Pen and Cage Culture of Fish, Tigbauan, Iloilo, Philippines, Feb. 11-22, 1979. pp. 107-117.

9. PATHOLOGY

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9-1. INTRODUCTION

Milkfish culturing is one of the most important forms of traditional aquaculture in Southeast Asia (Chao and Liao, 1984; Chen, 1984; Lee, 1984; Samson, 1984; Chong et al., 1984). The diseases afflicting milkfish, however, have received far less study than the diseases of other cultured fishes such as salmonids, catfish and anguilliform eels (Lio-Po, 1984). In many instances, milkfish diseases have not received adequate investigation to firmly establish their etiology, pathology, prevention and control. Thus, an increase in basic research effort is needed to strengthen the knowledge of milkfish diseases. This should be an important consideration to the milkfish industry.

This chapter reviews and discusses available information regarding milkfish diseases and their control. Although of considerable importance to milkfish culture, pests and predators are excluded because they do not directly result in disease of milkfish.

9-2. VIRAL INFECTIONS

There are no described viral diseases of cultured milkfish, however, an RNA virus has been found in cultured milkfish in Taiwan. In 1983-84, virus isolation studies were undertaken on milkfish fry from the western coastal area of Taiwan (162 fry samples from local fry dealers) and adult milkfish from cultured milkfish in southern Taiwan (165 pooled viscera samples). Tissue homogenates were inoculated into monolayer cultures of the established cell line of BF-2, as well as fibroblastic cells derived from the heart and the swim-bladder of milkfish. These have been subcultured more than 150 times over the past two years and appear to be established cell lines (Tung et al., unpublished). A virus was isolated in low titer but only from the milkfish fry sampled from the western coastal area of Taiwan. This virus appeared to be infectious pancreatic necrosis (IPN) virus, serotype Ab, based on serum neutralization and fluorescent antibody test findings. The isolated virus was found by intraperitoneal inoculation to be non-pathogenic to milkfish fingerlings and the glass eel, Anguilla japonica, and was not considered to be the cause of disease in milkfish (Tung, unpublished).

9-3. BACTERIAL DISEASES

Several bacterial diseases of milkfish have been recognized and bacteria -related mortality problems have been observed in milkfish culture for many years. The diseases known or suspected to be caused by bacteria include vibriosis (red spot disease, hemorrhagic septicemia), scale disease, fin rot and eye opacity. As research efforts increase, it is anticipated that additional bacterial diseases of milkfish will be recognized and described.

To date, the types of bacteria reported to be recovered from moribund milkfish or milkfish lesions have been facultative, opportunistic water inhabitants, which probably are ubiquitously distributed in the milkfish pond environment and occur throughout the natural range of milkfish. The species of bacteria reported, associated with bacterial diseases of milkfish, have been in the following three genera: Vibrio, Flexibacter and Aeromonas. Bacteria in these genera are known to be pathogenic to many species of fish.

1) Vibriosis or Red Spot Disease

Milkfish vibriosis or red spot disease is caused by Vibrio anguillarum (Huang, 1977; Tung et al., 1985). The causative bacterium is a gram negative, curved rod that grows well on media supplemented with additional NaCl. Trypticase Soy or Tryptose Agar with 1-3% NaCl or Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS) are suitable media for primary isolation of V. anguillarum from milkfish with vibriosis. Isolated bacteria can be identified by their biochemical and physiological characteristics. Both V. anguillarum I and II have been identified from milkfish (Huang, 1977; Chang and Kuo, 1983; Tung et al., 1985). The biochemical and other characteristics for these isolates are summarized in Table 1.

In Taiwan, mortality problems from vibriosis (red spot disease) have been most common in the winter months from November through March. During the past 15 years, the average cumulative mortality among milkfish fingerlings in overwintering ponds in Taiwan has been estimated to be 15% per year, with a peak of 70% in 1975 (Huang, 1977).

Table 1. Comparison of biochemical and physiological characteristics of *Vibrio anguillarum* strains isolated in Taiwan.

Characteristics	Bergey's Manual[a] *V. anguillarum* I[a]	II[a]	Strains isolated by Huang (1977)	Chang & Kuo (1983)	Tung et al. (1985)
Gram stain	-	-	-	-	-
Single polar flagellum	+	+		+	+
Cytochrome oxidase	+	+	+	+	+
0/129 sensitivity	S		S	S	S
Novobiocin sensitivity			S	S	S
Nitrate reduction	+	-	-	+	-
Gelatin liquifaction	+	+	+	+	+
Indole			+	d	-
Hydrogen sulfide			-	-	-
Methy red			-	-	-
Voges-Proskauer	+	-	-	+	-
Gas from glucose	-	-	-	-	-
Arginine dehydrolase	+	-	-	+	-
Lysine decarboxylase	-		-	-	-
Ornithine decarboxylase	-		-	-	-
Growth in % NaCl:					
3%	+	+			+
7%				-	d
10%	-	-		-	-
Utilization of:					
Arabinose	+	-	+	+	d
Cellobiose	+	-			-
Galactose	d	-		+	-
Glucose	+	+	+	+	+
Inositol	d	-	-	-	-
Lactose	-	-	-	-	-
Mannitol	+	-	+	+	-
Mannose	+	-	+	+	-
Salicin	-	-		-	-
Sorbitol	+	-		+	-
Sucrose	+	+	+	+	+
Trehalose	+	-			-
Xylose	-	-	-		-

a,b Bergey's Manual of Systematic Bacteriolcgy, Vol. 1 (1984);
+ = positive, - = negative, S = sensitive, d = different reactions given by different strains

Epizootics of vibriosis in milkfish fingerlings have frequently occurred in overcrowded ditch or canal holding areas where fingerling stocking rates are high, in many instances 1.3 kg/m^3 or more (Tsai et al., 1970). In most cases, an outbreak has occurred during a cold snap when the overwintering ditch has not received a regular flow of water from the outside pond because of low water temperature. Stressors, including low water temperature, oxygen depletion caused by high stocking density and poor water quality, are the major factors which predispose milkfish fingerlings to outbreaks of vibriosis (Tsai et al., 1970; Chen and Liu, 1972; and Huang, 1977).

Clinically, vibriosis usually occurs as an acute septicemia, although a more chronic form of the disease is known. Acutely affected milkfish are dark in color (blackish) and swim sluggishly at the surface of the water. Externally, milkfish suffering from vibriosis may have reddish spots and ulcers on the body, especially in the abdominal region; a swollen and inflamed anus (Fig. 1); and congestion at the base of the fins. Severely affected and dying milkfish may have extensive hemorrhage of the eyes, oral cavity and inner surface of the operculum (Fig. 2). The gills may be swollen and congested or pale, since some infected fish are anemic.

Internally, milkfish with vibriosis may have petechial hemorrhages and/or pale foci of the liver, kidney and intestinal serosa. The histopathological lesions are consistent with those for fish suffering from an acute hemorrhagic septicemia. For a detailed description of the histopathological finding for milkfish affected by vibriosis, the reader is referred to Tung et al. (1985).

2) Fin Rot

Fin rot is a common disease problem of milkfish in the Philippines (Lio-Po, 1984). Flexibacter columnaris has been identified from fin rot lesions of milkfish cultured in

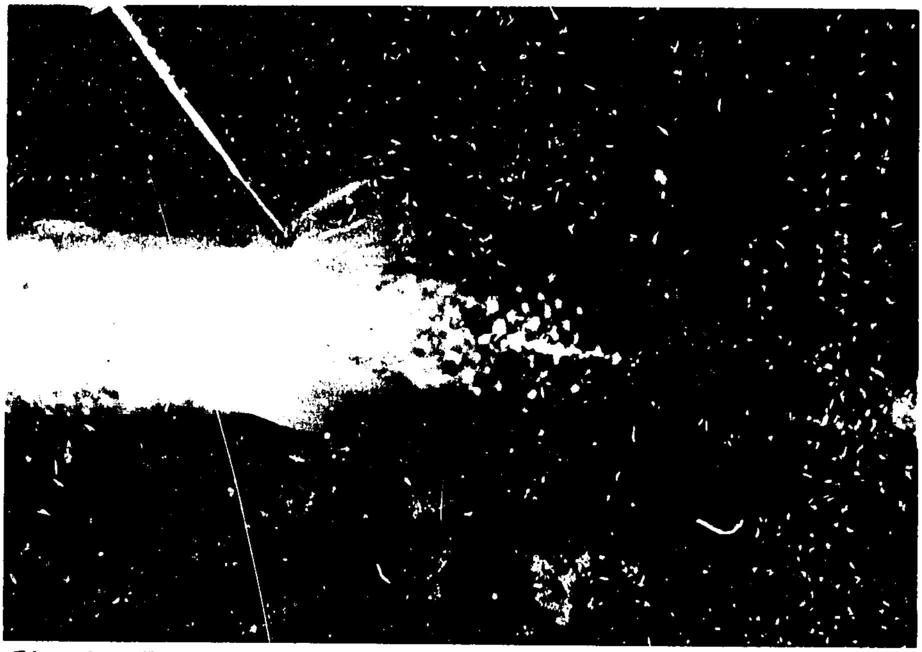

Fig. 1. The body surface of a milkfish showing reddish discoloration.

Fig. 2. Severe hemorrhagic lesion found on the inner surface of the operculum.

freshwater ponds in Taiwan (Tung, unpublished), and from juvenile milkfish reared in brackishwater ponds in the Philippines. Fin erosion-associated mortalities of milkfish, caused by an unidentified bacterium, have been reported in the Philippines (Lio-Po, 1984) and Hawaii (Timbol, 1974).

3) Other Bacteria-Related Problems

In earthen ponds in the Philippines, milkfish have been reported (Muroga et al., 1984) to suffer from eye abnormalities when polycultured with the Indian Prawn, _Penaeus indicus_. Clinically affected milkfish had unilateral to bilateral opacity of the cornea, exopthalmia and hemorrhage of the eye. Although _Vibrio parahaemolyticus_ or _V. alginolyticus_ were isolated from the affected fish and proven to be pathogenic through artificial inoculation tests to milkfish, the Japanese eel, _Anguila japonica_, and mice, these bacteria were thought to be secondary invaders. Muroga et al. (1984) concluded that the milkfish eye lesions were induced by some type of trauma, possibly by the prawns or mechanically induced, and then became infected by _Vibrio_ sp.

Mahadevan et al. (1978) claimed that _Vibrio parahaemolyticus_ is the etiologic agent which causes "scale disease" of cultured milkfish. Scale disease is a condition in which pus forms and causes the scales to protrude. _Vibrio_ sp. has been reported to have been isolated from muscle abscesses of milkfish spawners reared in floating cages in the Philippines. The abscesses were associated with hormone injection in these fish (Lio-Po, 1984).

Aeromonas hydrophila has occasionally been isolated and is believed to be the cause of systemic infections (general septicemia) of milkfish reared in freshwater ponds. This bacterium is a normal inhabitant of the freshwater aquaculture environment but may cause fatal systemic infections in heavily stressed fish exposed to poor water quality conditions or other stressors (Tung, unpublished).

9-3.2. CONTROL OF BACTERIAL INFECTIONS

Control of bacterial infection of milkfish has been attempted by several means, including vaccination, use of bacteriophage AS10, oral or bath treatment with antibiotics or bath treatment with disinfectants. To date, no single method of treatment has proven to be completely satisfactory, and research is needed to establish effective means of control for bacterial infections of milkfish.

1) Vaccination

Vaccination experiments using the HIVAV *V. anguillarum* bacterin[1] have been carried out with milkfish fingerlings (Song et al., 1980). The HIVAV bacterin produced some immunity to *V. anguillarum* which was found after one week following immersion exposure. This immunity lasted for at least three months at room temperature. The HIVAV bacterin was shown to be safe and effective for the vaccination of milkfish fingerlings as small as 0.38 gm body weight. However, evaluation of the vaccination efficacy in field trials indicated protection was not statistically significant (Lin et al., 1982). Additional research in milkfish defense mechanisms, immunology and vaccination methods is required before vaccination of milkfish can be shown to be an effective method in the control of milkfish vibriosis.

2) Bacteriophage

Bacteriophage AS10 has been isolated from the water in milkfish overwintering ditches. This bacteriophage has been shown to have the ability to infect and lyse a wide variety of bacteria including eighteen strains of *V. anguillarum* in Taiwan. The infectivity of *V. anguillarum* has been shown to be almost completely eliminated after four hours of exposure to AS10 at a multiplicity of infection (MOI) equal to one. Furthermore, in field studies, vibriosis of milkfish fingerlings was effectively arrested by applying and maintaining

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1. Manufactured by Tavolek, Inc., Redmond, Washington, U.S.A.

AS10 at 20 plaque forming units (PFU) per milliliter concentration. Nevertheless, careful evaluation of the use of mass application of bacteriophage must be undertaken. Such use must be shown to be safe, as well as effective, before this type of control method can be used in field applications.

3) Antibiotic Therapy

Various antibiotics have been used over the years to control milkfish vibriosis. Several of these compounds and their dosages are listed in Table 2. Lin and Ting (1983) conducted comparative studies with NF-Ueno-C20 (containing 20% of furazolidone), Monafuracin and sulfamonomethoxine. They reported that NF-Ueno-C20 gave the best therapeutic results at 10 ppm immersion. Milkfish treated with NF-Ueno-C20 had 100% and 75% survival when challenged by immersion and intraperitoneal injection of *V. anguillarum*, respectively. Less effective in this study was Monafuracin, followed by sulfamonomethoxine. Tung et al. (1985) found oral administration of trimethoprim-sulfonamide (at a ratio of 1:5) an effective treatment to control vibriosis in milkfish. Lio-Po (1984) reported that a daily bath with oxytetracycline for five consecutive days could arrest vibriosis.

In Taiwan, antibiotic resistant strains of *Vibrio anguillarum* have apparently become commonplace. Whereas *V. anguillarum* isolated in 1977 was highly susceptible to chloramphenicol and tetracycline, in 1984-85 the isolates were resistant to most antimicrobial agents tested (Huang, 1977; Tung et al., 1985). The results of drug sensitivity tests with the Taiwan isolates are listed in Table 3.

Some success has been obtained in the treatment of vibriosis by use of a disinfectant bath. Lin and Ting (1983) reported the use of San-O-Fec-50 (containing 50% quarternary ammonium compound), IOFEC-80 (7% available iodine) and iodophores (20% available iodine) at dosages of 0.5 to 1.0

Table 2. Antibiotics and dosages reported for the control of vibriosis of milkfish.

Antibiotics	Treatment	Reference
NF-Ueno-C20 (containing 20% furazolidone)	10 ppm, bath	Lin (1983)
Monafuracin (nitrofurazone)	0.1 ppm, bath	Lin and Ting (1983)
Sodium sulfamonomethoxine	100 ppm, bath	Lin and Ting (1983)
Furanace	1.6-3.1 mcg/ml, bath	Pearse et al. (1974)
	1 ppm, daily bath for 5 days	Muroga, unpublished
Trimethoprim-sulfonamide (1:5) TMP-SDX	Oral	Tung et al. (1985)
Oxytetracycline	Daily bath for 5 days	Lio-Po (1984)

ppm, 0.4 ppm and 0.15 ppm, respectively, to control bacterial disease. Lio-Po (1984) has reported that introducing freshwater into culture tanks could also arrest infection.

9-4. MYCOTIC DISEASES

While several reports of fungal infections of milkfish have been reported from the Philippines and Hawaii, identification of the fungal agent or demonstration of its pathogenicity has not been reported. Diagnosis of fungal infection of milkfish has been based on the finding of fungal hyphae within lesions of milkfish. A 3% prevalence of fungal infection in sampled milkfish fry has been reported (Anon., 1973). In another case, a batch of juvenile milkfish suffered an 8% mortality attributed to fungus infection. Fungal infection was implicated in market-sized milkfish with opacity of the eye ("milky eye disease") and milkfish breeders with similar opaqueness of the eye membrane, found to occur within 24 hours after the fish were caught by trap and released into holding pens. The affected fish apparently recovered spontaneously without treatment within a few days

Table 3. Drug sensitivity of *Vibrio anguillarum* isolated from diseased milkfish in Taiwan.

Drug	Huang (1977)	Tung et al. (1985)
Ampicillin (10 mcg)		-
Carbenicilline (100 mcg)		-
Chloramphenicol (30 mcg)	+++	-
Chlortetracycline (30 mcg)		++
Colistin (50 mcg)		d
Doxycycline (30 mcg)		++
Erythromycin (15 mcg)		-
Furadoine (300 mcg)		+++
Furazolidone (300 mcg)		+++
Gentamycin (10 mcg)		d
Kanamycin (30 UI)	+	d
Nalidixic acid (30 mcg)		-
Neomycin (5 mcg)		-
Nitrofurantoin (300 mcg)		-
Novobiocin (5 mcg)	+	d
Oleandomycin	-	
Oxytetracycline (30 UI)		++
Penicillin (100 mcg)	-	-
Polymyxin B (300 U)		-
Streptomycin (10 mcg)		-
Tetracycline (30 mcg)	+++	d
Trimethoprim-Sulfamethoxazole (1.25 mcg + 23.75 mcg)		+++

+++ - + = highly sensitive to sensitive

- = resistant

d = different reactions given by different strains

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(Anon., 1976). Timbol (1979) reported fungal infection associated with scale loss in milkfish in Hawaii. Treatment was attempted with satisfactory results using potassium permanganate (10 ppm), pyridyl mercuric acetate (2 ppm) and malachite green (10 ppm). Delmendo (1978) reported that fungal infection of fry and fingerling milkfish usually occurs in the winter.

9-5. PARASITIC INFECTIONS

Milkfish are known to be suitable hosts for a number of parasites. Parasites of importance to cultured milkfish are discussed below.

Of the Isopora, Rocinella typicus and Ichthyoxyenous are known to be infectious for, and cause mortality of all sizes of milkfish (Velasquez, 1979). These parasites have a direct life cycle and apparently the potential to reproduce rapidly leading to heavy infections of susceptible milkfish. Recommended control methods include: removal of all the infected fish and transfer of noninfected individuals to uncontaminated ponds, draining of infected ponds and applying lime and leaving the ponds empty for several weeks before restocking with milkfish (Velasquez, 1979).

In the Philippines, cultured milkfish may be infected by zoonotic species of heterophyid digenetic trematodes including Haplorchis varium, H. yokogawai and Procerovum calderoni. Milkfish serve as second intermediate host for these flukes as snails. Final hosts are mammals, including humans. Thus these parasites are of significance as they can infect humans if the fish flesh is consumed raw or improperly cooked (Velasquez, 1979).

Caligid exoparasites have been reported to infect cultured milkfish in the Philippines (Velasquez, 1984) and Taiwan (Huang and Hung, 1964; Lin, 1968; Lin, 1982). In the Philippines, Caligus patulus is frequently found attached to milkfish and can cause death of these fish (Velasquez, 1984). In Taiwan, C. orientalis is known to cause problems when fingerling milkfish are crowded in overwintering ditches. Apparently, if the fingerlings remain healthy and active, the caligids are unable to effectively attach to the fish. However, if the fingerlings become sluggish and weak during sudden cold spells, the caligids are able to attach and heavy infections can result in death of the fish. Heavily infected milkfish may be dark colored, swim sluggishly near the surface and be emaciated. Occasionally, infested fish may swim and jump vigorously. Caligid feeding sites often become hemorrhagic and may provide a portal of entry to secondary infection by bacteria or fungi.

Treatment with a 1-3% formalin bath or Neguvon is recommended in the Philippines for Caligus sp. infection (Velasquez, 1984). In Taiwan, treatment with 0.125 ppm Masoten (Dylox, Neguvon) at two week intervals has been found effective (Lin, 1982).

Lernaea cyprinicea, or "anchor worm", has been found to infect milkfish fingerlings reared in fishpens in Laguna de Bay. A 3-5% salt bath was found to be an effective treatment (Velasquez, 1979)

9-6. MISCELLANEOUS DISORDERS

Several miscellaneous disorders or pathological conditions have been reported from cultured and wild milkfish. Of these, the most significant appear to be caused by exposure to stressful conditions. Indeed, most documented outbreaks of disease in milkfish populations have been associated with exposure to one or more predisposing stressors. All of the following have been implicated as stressors of milkfish: overcrowding, drastic change in weather or water temperature, trauma and fright caused by rough handling during transport, high levels of ammonia, starvation and hypoxic water conditions (Rabanal et al., 1951; Tsai et al., 1970; Chen and Liu, 1972; Duncan, 1974; Huang, 1977; Delmendo, 1978; Jumalon, 1979; Cruz, 1981; Cruz and Enriquez, 1982; Lin, 1982; Lio-Po et al., 1982; Lio-Po, 1984).

Stressed milkfish develop black discoloration of the back (dorsal surface). Smith and Ramos (1976) reported the early detection of stress in milkfish can be determined by demonstration of occult hemoglobin in the mucous of the stressed fish. The hemoglobin test method uses commercially available hemoglobin test strips.

Pesticides and toxic pollutants from industrial or livestock effluent may act as stressors at low levels (Palma-Gil et al., unpublished) or be acutely toxic and cause mass mortalities at higher levels (Liu et al., unpublished).

Lio-Po et al. (1983) reported mortalities of 8 to 60% of milkfish fry from gas bubble disease. Gas bubbles were observed in the abdominal cavity and visceral organs of affected fish, suggesting that gas supersaturation was the cause of the problem.

Eye damage has been frequently observed in milkfish and is usually associated with trauma following handling or transport. Secondary infection of traumatized eyes by bacteria and/or fungi has been a common sequela. Affected fish may recover spontaneously or fish with damaged eyes may eventually die.

Tamse et al. (1983) reported necrosis of the iris, slight thickening of the lens capsule and detachment and destruction of the retina of milkfish fingerlings in nutritional requirement tests. However the relationship of these abnormalities to deficiency of specific nutrients was not established in the study.

Smith (1978) reported gastritis affecting 25 to 65% of wild milkfish weighing 2-8 kg that were collected from certain locations in Hawaii. The author speculated that the highest incidence of gastritis appeared to be caused by a combination of polluted water and possible genetic susceptibility to gastritis as a result of inbreeding.

Smith (1980) reported a case of spontaneous thrombus in an adult milkfish, which occluded the lumen between the bulbous arteriosus and the ventricle preventing or limiting blood flow from the heart. This fish grew and fed well for some time and was reported to have died suddenly. The author speculated that a variety of factors commonly encountered under aquacultural conditions could lead to the formation of such lethal clots.

9-7. SUMMARY

There are no described viral diseases of cultured milkfish, however, milkfish fry caught from the western coast of Taiwan were found to carry infectious pancreatic necrosis virus (Ab type) in low titer. The significance of the virus to milkfish is not known. A number of milkfish diseases caused by bacterial organisms from the genera Vibrio, Flexibacter and Aeromonas have been recognized. Among these, the most significant mortality has been associated with V. anguillarum infection. Both V. anguillarum I and II have been isolated from diseased milkfish. Vaccination experiments of V. anguillarum bacterin and the utilization of bacteriophage AS10 to control vibriosis in milkfish has been initiated in Taiwan. However, further research in these areas is necessary to achieve safe and efficient preventive measures against disease. The oral or bath treatments with antibiotics or disinfectants are used with some success. Although a few fungi associated with mortality or eye and skin infections have been observed in the Philippines and Hawaii, none of the fungal etiology has been identified thus far. Several parasites including copepods and heterophyid flukes are known to exist in the Philippines, whereas milkfish fingerlings in Taiwan may suffer from attacks of

Caligus orientalis in overwintering holding ditches. Several miscellaneous disorders of milkfish have also been discussed.

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REFERENCES

Anonymous. 1973. Parasites. Inland Fisheries Project Technical Report. Freshwater Aquaculture Center (FAC), University of the Philippines, No. 4: 31-34.

Anonymous. 1976. Preliminary study on the artificial reproduction of bangus (Sabalo). Inland Fisheries Project Technical Report. Freshwater Aquaculture Center (FAC), University of the Philippines, No. 9: 66-80.

Chang, C.Y. and G.H. Kuo. 1983. Study on serotypes of Vibrio anguillarum isolated from cultured fishes in Taiwan. CAPD Fish. Series, No. 9: 22-30.

Chao, N.H. and I.C. Liao. 1984. Status and problems of propagation of marine finfish in Taiwan. In: I.C. Liao and R. Hirano (Eds.) Proceedings of ROC-JAPAN Symposium on mariculture. Tungkang Marine Laboratory, Tungkang, Pingtung, Taiwan, 1: 33-49.

Chen, H.C. and C.Y. Liu. 1972. Ecological study of milkfish wintering pond. JCRR Fish. Series, No. 12: 35-49.

Chen, T.P. 1984. Mariculture in Taiwan with reference to sea ranching. In: I.C. Liao and R. Hirano (Eds.) Proceedings of ROC-JAPAN Symposium on mariculture. Tungkang Marine Laboratory, Tungkang, Pingtung, Taiwan, 1: 13-17.

Chong, K.C., A. Poernomo and F. Kasryno. 1984. Economic and technological aspects of the Indonesian milkfish industry. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc. Manila, Philippines. pp.

199-215.

Cruz, E.R. 1981. Acute toxicity of un-ionized ammonia to milkfish (Chanos chanos) fingerlings. Fish Res. J. Philipp. 6: 33-38.

Cruz, E.R. and G.L. Enriquez. 1982. Gill lesions associated with acute exposure to ammonia. Nat. and Appl. Sci. Bull. 34: 1-13.

Delmendo, M.N. 1978. An overview of fish diseases and their control in aquaculture in the Pacific Region. Paper prepared for the Workshop on Disease of Fish Cultured for Food in Southeast Asia. Cisarua, Bogor, Indonesia, November 28 - December 1, 1978. 18 p.

Duncan, B.L. 1974. Diseases of cultured fish, methods of investigation and data reporting. Inland Fisheries Project, Bureau of Fisheries, Philippines. 16 p.

Huang, T.L. and C.P. Hung. 1964. Control of fish louse, Caligus sp. in wintering ponds. J. Chinese Fish. 139: 11-12.

Huang, Y.H. 1977. Preliminary report of the studies on bacterial disease of milkfish, Chanos chanos during winter. JCRR Fish. Series, No. 29: 50-54.

Jumalon, N.A. 1979. Acute toxicity of un-ionized ammonia to milkfish Chanos chanos (Forsskal) fry. SEAFDEC AQD, Philippines, Quarternary Res. Rpt. 3: 10-14.

Lee, C.S. 1984. The milkfish industry in Taiwan. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc. Manila, Philippines. pp. 183-199.

Lin, C.L. 1982. Milkfish diseases and control in overwintering period. Fisherman J. 5(6): 42-44.

Lin, C.L., Y.Y. Ting and Y.L. Song. 1982. Proceeding evaluation of HIVAX Vibrio anguillarum bacterin in the vaccination of milkfish (Chanos chanos) fingerlings. CAPD Fish. Series, No. 8: 80-83.

Lin, C.L. and Y.Y. Ting. 1983. Effect of chemotherapeutic agents by medicated bath on the red spot disease (vibriosis) of milkfish (Chanos chanos). CAPD Fish. Series, No. 9: 51-61.

Lin, S.Y. 1968. Milkfish farming in Taiwan. A review of practice and problems. Taiwan Fisheries Research Institute Fish Culture Report, No. 3: 1-63.

Lio-Po, G.D. 1984. Diseases of Milkfish. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc. Manila, Philippines. pp. 145-155.

Lio-Po, G.D., J.P. Pascual and J.G. Santos. 1982. Country report on Philippine fish quarantine and fish diseases. In: F.B. Davy and A. Chouinard (Eds.) Fish Quarantine and Fish Diseases in Southeast Asia. Report of a workshop held in Jakarta, Indonesia, December 7 - 10, 1982. IDRC, Indonesia. pp. 35-46.

Lio-Po, G.D., R.C. Duremdez and A.R. Castillo, Jr. 1983. An investigation of gas bubble disease occurrence among milkfish, Chanos chanos (Forsskal) fry. Presented at the Second International Milkfish Aquaculture Conference, Iloilo City, Philippines, October 4 - 8, 1983. 41 p.

Mahadevan, S., T. Pillai and D. Samuel. 1978. Diseases of finfishes and shellfishes cultivated in the coastal waters of India. Paper prepared for the Workshop on Disease of Fish Cultured for Food in Southeast Asia. Cisarua, Bogor, Indonesia, November 28 - December 1, 1978. 8 p.

Muroga, K., G. Lio-Po, C. Pitongo and R. Imada. 1984. Vibrio sp. isolated from milkfish (Chanos chanos) with opaque eyes. Fish Pathol. 19: 81-87.

Pearse, L., R.S.V. Pullin, D.A. Conroy and D. McGregor. 1974. Observation on the use of furanace for the control of vibrio disease in marine flatfish. Aquaculture 3: 295-302.

Rabanal, H.R., H.R. Montalban and D. Villaluz. 1951. The preparation and management of bangus fishpond nursery in the Philippines. Philipp. J. Fish. 1: 3-35.

Samson, E. 1984. The milkfish industry in the Philippines. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc. Manila, Philippines. pp. 215-228.

Smith, A.C. 1978. Pathology and biochemical genetic variation in the milkfish, Chanos chanos. J. Fish Biol. 13: 173-177.

Smith, A.C. 1980. Formation of lethal blood clots in fishes. J. Fish Biol. 16: 1-4.

Smith, A.C. and F. Ramos. 1976. Occult hemoglobin in fish skin mucus as an indicator of early stress. J. Fish Biol. 9: 537-541.

Song, Y.L., S.N. Chen, G.H. Kuo, C.L. Lin and Y.Y. Ting. 1980. HIVAX Vibrio anguillarum bacterin in the vaccination of milkfish (Chanos chanos) fingerlings. CAPD Fish. Series No. 3: 101-108.

Tamse, C.T., F. Piedad-pascual and M.C. de la Cruz. 1983. Some histopathological observations on the opaque eyes of milkfish Chanos chanos (Forsskal). Paper presented at the Second International Milkfish Aquaculture Conference, Iloilo City, Philippines, October 4 - 8, 1983. 5 p.

Timbol, A.S. 1974. Observations on the growth of young bangus, Chanos chanos (Forsskal) on two types of pelleted food. Philipp. J. Sci. 103: 199-206.

Tsai, S.C., H.S. Lin and K.Y. Lin. 1970. Some factors regarding the mortality of milkfish during overwinter period. Aquaculture 1: 9-30.

Tung, M.C., S.S. Tsai and S.C. Chen. 1985. Study on Vibrio anguillarum infection in cultured milkfish, Chanos chanos, in Taiwan. COA Fish. Series, No. 14 (in press).

Velasquez, C.C. 1979. Pests/Parasites and diseases of Chanos chanos (Forsskal) in the Philippines. Technology Consultation on Available Aquaculture Technology in the Philippines. In: SEAFDEC/PCARR (Philippine Council for Agriculture and Resources Research) Proceedings, Tigbauan, Iloilo, Philippines. pp. 65-67.

Velasquez, C.C. 1984. Pests/Parasites and diseases of milkfish in the Philippines. In: J.V. Juario, R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc. Manila, Philippines. pp. 155-161.

10. ECONOMIC ASPECTS OF MILKFISH FARMING IN ASIA

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10-1. INTRODUCTION

For centuries, milkfish has been the major finfish species cultured in Taiwan, the Philippines and Indonesia. In 1983, the milkfish industry in these countries utilized about 472,000 hectares of brackish and freshwater area and produced approximately 280,000 metric tons of fish (Table 1).

A comprehensive evaluation of milkfish economics is difficult because reliable, up-to-date economic data on the milkfish industry are not available in major producing countries. Most of the economic research conducted in the 1970's (Guerrero and Darrah, 1974; Librero et al., 1976, Lobrero and Nicholas, 1977; Shang, 1976a, 1976b, 1976c; Ramirez, 1978; Wiratno, 1978) is now out-of-date. Neverthe-

Table 1. Milkfish production in Taiwan, the Philippines and Indonesia for selected years.

Country	Year				
Taiwan	1970	1975	1979	1982	1983
Brackishwater area (ha)	16,738	18,115	19,654	20,345	21,300
Area used for milkfish production	16,360	16,800	15,345	14,563	14,740
Milkfish production (tons)	27,857	33,490	32,034	23,416	27,964
Milkfish as % of total production from brackishwater area	88	75	61	46	45
Freshwater ponds	NA	NA	NA	5	7
Milkfish production (tons)	NA	NA	NA	6,104	9,021
Philippines	1970	1975	1981	1982	1983
Brackishwater area (ha)	168,118	176,032	195,832	195,832	196,269
Production (tons)*	96,461	106,461	153,382	162,432	165,396
Freshwater area (ha)	NA	NA	25,000	NA	NA
Production (tons)	NA	NA	55,736	NA	NA
Indonesia	1972	1977	1981	1982	1983
Brackishwater area (ha)	178,297	174,605	198,210	208,695	242,308
Production (tons)*	32,800	48,641	61,041	73,300	81,506
Milkfish as % of total production from brackishwater ponds	64	56	54	57	60

* Includes all species. Milkfish as percentage of total production was estimated about 90% in 1981 in the Philippines. The same percentage was used for 1982 and 1983.

Source: Smith and Chong, 1984a; Chong et al., 1984; Taiwan Fisheries Bureau, 1984; Bureau of Fisheries and Aquatic Resources, the Philippines, 1984; Department of Fisheries, Indonesia, 1985.

less, despite the above limitations and gaps in data, it is possible to draw some inferences from the information available in each country, especially from recent studies: Chong et al., 1982, 1984; Smith and Chong, 1984a, b; Lee, 1983; Jackson, 1983; and Samson, 1984.

10-2. TRENDS OF DEVELOPMENT

Except for Taiwan, the production of milkfish in the Philippines and Indonesia has been increasing in area and quantity.

10-2.1. TAIWAN

Despite the overall expansion of aquaculture in Taiwan, the relative contribution of milkfish has decreased significantly. Of the total aquaculture sector in Taiwan, the share of milkfish production has declined dramatically, from 39% in 1970 to 15% in 1983. During the same period, milkfish production as a percentage of total production from the brackishwater area declined from 88% to 45%. The brackishwater area used for milkfish production decreased between 1970 and 1983 but the recent decline was partially offset by use of freshwater ponds for milkfish production.

The lack of growth in the milkfish industry in Taiwan is due mainly to the declining profitability of milkfish production. The real (deflated) costs of major inputs have been increasing during the past decade, while the real wholesale prices of milkfish have been stable (Smith and Chong, 1984a). Shang (1976b) reported an 18% rate of return on operation capital for the average Taiwanese milkfish farmer in 1972, while Lee (1983) indicated a lower rate of 10%, less than the opportunity cost of capital. In response to the cost-price squeeze, three major changes in brackishwater production techniques have recently been evidenced (Lee, 1983; Smith and Chong, 1984a): (1) many producers began raising other species with higher profit, such as the Tiger prawn (Penaeus monodon), in brackishwater ponds formerly used for milkfish; (2) some producers specialized in the production of milkfish fingerlings as baitfish for tuna longliners and realized significantly higher profits than with the production of market-size milkfish. Increased fuel costs and decreased tuna prices have mitigated the demand for milkfish, however; and (3) some producers have converted to the deep-water pond production method because of higher profits.

10-2.2. PHILIPPINES

Milkfish has been the major species cultured in the Philippines and it remains the dominant species today. The production area and the quantity of production have been increasing in brackishwater ponds and in freshwater fish pens, especially the latter in Laguna de Bay (up to 1984). The relative contribution of milkfish to total aquaculture production in the Philippines is declining with the recent rapid expansion of freshwater aquaculture. Tilapia has become increasingly popular with producers and consumers and is competing with milkfish in local markets (Smith and Chong, 1984a).

The increasing average productivity of milkfish ponds indicates that culture practices have improved in general. In the mid-1970s, several economic studies (Shang, 1976b; Librero et al, 1976; Nicholas and Librero, 1978) reported that brackishwater pond operators earned, on the average, a positive net revenue. A later study, based on the 1978 crop year (Chong et al., 1982), showed a slightly higher profit than the previous studies, but small farms of less than 6 ha incurred losses in most provinces surveyed. Many milkfish pond operators have complained that their profits are declining (Smith and Chong, 1984a). In Metro Manila, retail prices for milkfish have not increased significantly since 1979, in spite of declining fishery supplies in the country and population growth. In fact, the real (deflated) price in 1983 was actually 21% lower than it was in 1974 and 30% lower than in 1979. Milkfish fry and fertilizer prices have continued to increase (Smith and Chong, 1984a). In general, milkfish producers, like their Taiwanese counterparts, are caught in a cost-price squeeze with declining profits. In response to the cost-price squeeze, increasing numbers of milkfish farmers practice polyculture with shrimp and/or shrimp monoculture due to the high price and high profit of shrimp culture.

10-2.3. INDONESIA

Both the area and production of brackishwater ponds have been increasing but the share of milkfish production as a percentage of total production from brackishwater ponds has been declining. There is a relative shift towards species other than milkfish. Tilapia and shrimp production in brackishwater ponds have each increased at substantially higher rates. The recent ban on shrimp trawling in coastal waters has had a significant impact on milkfish farming. With expected reductions in the supply of shrimp from capture fisheries and increased prices in export markets, shrimp farming in brackishwater should become increasingly attractive.

The average production of milkfish per ha is about half of that in the Philippines and only one-sixth of that in Taiwan. The majority of farms use extensive systems with low stocking rates and without much purchased input. Shang (1976a, 1976c) indicated that profits from milkfish farms can be increased if fertilizer and supplementary feeds are used and proper stocking manipulation is applied. It is also evidenced in recent studies (Wiratno, 1978; Jackson, 1983; Chong et al., 1984) that milkfish farms are, in general, operated at far below their maximum potential. About 45% of total milkfish farms have not used any type of fertilizer (Chong et al., 1984). Many governmental actions have been attempted to encourage milkfish operators to use more inputs, but progress is very slow. Low survival rates and high risks have been mentioned as reasons for low inputs (Wiratno, 1978). A recent study indicated that the increase in input prices such as for milkfish fry, rice bran and fertilizer was greater than that of market-size milkfish (Smith and Chong, 1984a). The intensification project in certain areas has depressed the price of milkfish and many milkfish farmers have cut back on production (Chong et al., 1984). It is likely that Indonesian milkfish farmers are being caught in a cost-price squeeze similar to their Taiwanese and Philippine counterparts.

10-3. MAJOR FACTORS AFFECTING PRODUCTION ECONOMICS

10-3.1. MAJOR COST ITEMS

The production costs of milkfish are mainly dependent on local environmental and economic conditions, farm sizes and culture systems. Despite the lack of up-to-date cost studies in these three major producing countries, the information in Table 2 provides some indication of the major cost items in each country.

In Taiwan, fry is the most important cost item, followed by fertilizer/feed and labor. Milkfish fry are collected manually from the coastal waters in all three major producing countries, but the annual fry catch in Taiwan is inadequate to meet fishpond stocking requirements. The Taiwanese milkfish industry faces a chronic shortage of fry and must rely on imports from the Philippines and Indonesia for almost half of its annual requirements. The relatively high cost of fry and high stocking rate make fry the most important cost item. The availability of fry in the Philippines and Indonesia fluctuates seasonally and regionally. There is a need for inter-country shipment although there is no significant fry shortage on the whole. Milkfish fry in these two countries is also a major cost item. A stable and adequate supply of fry at a reasonable price is necessary for

Table 2. Major cost items of milkfish production in Taiwan, the Philippines and Indonesia.

Item	Taiwan[a]	Philippines[b]	Indonesia[c]
Fry	40%	15%	19%
Feeds/fertilizer	27	25	6
Pesticides	3	2	5
Labor	20	27	31
Water/electricity	1	-	-
Maintenance	-	-	-
Land rent	2	-	23
Interest	4	-	-
Taxes	-	-	2
Depreciation	3	-	14
Miscellaneous	1	31	-

[a] Lee, 1983.
[b] Chong et al., 1982. Average of all size farms
[c] Wiratno, 1978.

industry expansion. It is desirable that milkfish be reproduced in captivity.

In the Philippines and Indonesia, labor is the major cost item. Production function analyses in the Philippines (Chong et al, 1982b) and Indonesia (Jackson, 1983) indicate that milkfish farms in these two countries use more labor than they need and that profits could be increased by using fewer laborers.

10-3.2. FARMING INTENSITY

In 1982-83, the average productivity of brackishwater milkfish ponds in Taiwan was about 1,900 kg/ha compared to about 820 kg/ha in the Philippines and 340 kg/ha in Indonesia. The relatively high productivity of milkfish ponds in Taiwan is primarily due to the higher stocking rate and greater use of fertilizer/feed. Since the growing season of milkfish in Taiwan is limited to about seven months a year and suitable land for milkfish culture is limited, intensive farming is usually practiced. Pond production is increased by stocking fish of different sizes (overwintered fingerlings and new fry) and periodic harvest of marketable fish and restocking of fry. Therefore, the total fish population or biomass at any one time is near the optimum carrying capacity of the pond. If this method was not employed, the yield per hectare in Taiwan would not be better than that of the Philippines where the growing season occurs year round. Milkfish farmers in Taiwan used more feed and fertilizer than those in the Philippines and Indonesia. This is one of the major factors that permits higher stocking rates and hence the high yield in Taiwan.

Shang (1976a) reported that intensive milkfish farming in the Philippines and Indonesia increases profit compared to that of extensive operations. A recent production function analysis using cross-section data in the Philippines (Chong et al., 1982b) and Indonesia (Jackson, 1983) indicates that the average milkfish farmer in these two countries can increase output and return by increasing the stocking rate of fry and the application rate of fertilizers. These conclusions, however, must be interpreted carefully due to the relative change in input costs and output prices. Additional input is economically feasible only if the added value is in excess of additional costs.

10-3.3. DEEPWATER PONDS

Some milkfish producers in Taiwan have recently deepened brackishwater ponds from their traditional 10-30 cm depth to 2-3 meters, and this, coupled with commercial feed application, higher stocking rates (20,000 pieces/ha/yr of 15-18 cm size) and pond aeration, has increased production. The average yield can be increased from the previous 2 tons/ha/yr to over 10 tons (Smith and Chong, 1984a). Using this "deepwater method", profits can also be substantial, surpassing even those of shrimp farming. The analysis of the input-output relationships of the Philippines milkfish production (Chong et al., 1982a) indicates that economic benefits can also be increased by using more inputs in deeper ponds.

10-3.4. FARM SIZE

The milkfish economic analysis in Taiwan (Lee, 1983) indicates that farm income and resource productivity are closely related to farm size (Table 3). The larger the farm size, the lower the cost of production and the higher the farm income and resource productivity. This is also evidenced with milkfish farming in the Philippines (Carandang and Darrah, 1973; Chong et al., 1982;). Returns from major inputs (land, labor, capital and management) can be increased with an increase in farm size.

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Table 3. Economics of scale of milkfish production in Taiwan.

Item	Farm Size (ha)		
	<3	3-10	>10
Farm income* (NT$/ha)	5,194	7,399	10,520
Land productivity (NT$/ha)	96,625	99,886	103,195
Labor productivity (NT$/man-day)	823	1,195	1,454
Capital productivity (NT$/NT$)	1.06	1.08	1.12

* farm receipts minus production costs.

Source: Lee, 1983.

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10-3.5. POLYCULTURE

The most important consideration in polyculture is the probability of increasing production and hence profit by better utilization of pond environments. For example, in the Philippines and Indonesia, the net income from polyculture of milkfish (primary species) and shrimp (secondary species) increases twofold compared to monoculture (Shang, 1976a). This explains why milkfish ponds are being used increasingly for polyculture with shrimp (P. monodon).

10-4. MARKET POTENTIAL

The milkfish industry has the capability for expansion only if there is a potential market to absorb increased production at price levels that provide the producers a reasonable profit.

In Taiwan, milkfish is mainly sold for domestic consumption. The inability of the Taiwanese domestic market to absorb increased production of milkfish is a major constraint to the expansion of the milkfish industry. Between 1961 and 1983, there has been a dramatic fourfold increase in real per capita income in Taiwan. Until 1970, annual per capita fish consumption also increased, but since then, it has leveled off at about 37-39 kg, while consumption of other competing protein products, such as meat, eggs and vegetables, has continued to increase (Lee, 1983; Smith and Chong, 1984a). As disposable income increased, consumer preference for meat over fish emerged. Some experienced observers also believe that an increasing proportion of milkfish consumers have shifted to other, higher-priced species.

In the Philippines, annual per capita fish consumption declined from 38 kg in 1970 to about 20 kg by 1980 (Smith and Chong, 1984a). Historically, milkfish has been a first-class fish in the Philippines, priced higher than many marine species. Real per capita income in the Philippines has declined by more than 30% since 1972 due to the high rate of inflation. A continuing decline in real per capita income will result in an even greater reduction in consumption of traditional first-class fish such as milkfish. Cheaper species will be in greater demand.

Milkfish markets in Indonesia are largely localized because of the distance separating the production area and markets (Chong et al., 1984). The production from some islands cannot be shipped at a profit to Jakarta's markets with prevailing market prices and transportation costs. With localized markets, any effort to encourage farmers to increase their output runs into marketing problems, because the increased production cannot be disposed of without depressing the price.

10-5. SUMMARY AND CONCLUSION

Various problems and risks confront the milkfish industries in the three major producing countries. The cost-price squeeze with resultant declining profits is the major concern of the milkfish industry. There is a tendency among milkfish producers in the major producing countries to shift to other, more profitable species.

There is certainly a potential for making milkfish farming more profitable, i.e., through a reduction in the average cost of production and by eliminating market inefficiencies. For example, fry and fertilizers make up the bulk of production costs. Combining these inputs with land and labor in a cost-effective manner is a challenge to the more progressive producers. Individual producers in the Philippines and Indonesia may benefit by increased use of supplementary inputs. Similarly, the deepwater system used by Taiwanese farmers is one way to reduce average production costs and thus increase profits. Polyculture would also increase profits. If milkfish fry can be produced in hatcheries at a reasonable cost, it would benefit milkfish producers.

Many milkfish farmers use little or no purchased input mainly because of high risks such as flood, typhoons, low survival rates, high input costs, etc. In these cases, risk reduction or compensating measures such as low credit for operating capital, infrastructure improvements to reduce flood risk, improvements of fry collection and distribution techniques, subsidies on some of the purchased inputs such as fertilizer, and control of water pollution in coastal areas will be needed along with new culture techniques.

The economic feasibility of all the measures mentioned above, however, must be carefully evaluated. The blueprint for a profitable operation in one country is no guarantee that the application would succeed in another socio-economic setting. Nevertheless, transmitting information on economically viable options to the private sector is one of the greatest needs of the milkfish industries of the Philippines and Indonesia.

Much attention has been focused on means for increasing total production and yield, without much concern for the disposition of the increased production at remunerative prices to producers. Market constraints are emerging as the major problem facing the milkfish industry in Asia. In Taiwan, consumers have gradually shifted away from milkfish consumption due to the increase in disposable personal income and the change in consumer preference. By contrast, consumers in the Philippines have a tendency to shift to cheaper fish because of the drop in real per capita income. Localized markets and high shipping costs have hindered production growth in Indonesia. Improvements in transportation and processing, market development and promotion for new products (boneless, canned milkfish, etc.), for new uses (such as baitfish in Taiwan) and for new markets (export market) will benefit the industry. Again, the economic potential and feasibility of such options need to be evaluated.

Nearly all of the available economic studies on milkfish deal with micro-economic analysis. From the socio-economic point of view, macro-economic studies are needed.

In conclusion, the growth of the milkfish industry in the major producing countries depends, to a large extent, on

(1) the relative profitability between milkfish and alternative species production, and (2) the availability and price of competing products in the marketplace. Improvements in production and marketing efficiencies will certainly benefit the industry.

REFERENCES

Bureau of Fisheries and Aquatic Resources. 1984. Fisheries Statistics of the Philippines, 1982. Manila, Philippines.

Carandang, F.L. and L.B. Darrah. 1973. Bangus production costs. Department of Agriculture and Natural Resources, Quezon City, Philippines.

Chong, K.C. and M.S. Lizarondo. 1982. Input-output relationships of Philippine milkfish aquaculture. In: Aquaculture Economics Research in Asia. IDRC, Ottawa, Canada and ICLARM, Manila, Philippines.

Chong, K.C., M.S. Lizarondo, V.F. Holazo and I.R. Smith. 1982a. Inputs as related to output in milkfish production in the Philippines. Bureau of Agricultural Economics, Fishery Industry Development Council and ICLARM, Manila, Philippines.

Chong, K.C., I.R. Smith and M.S. Lizarondo. 1982b. Economics of the Philippines milkfish resource system. The United Nations University, Tokyo, Japan.

Chong, K.C., A. Poernomo and F. Kasryno. 1984. Economic and technological aspects of the Indonesia milkfish industry. In: J.V. Juario, J.R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc., Manila, Philippines.

Department of Fisheries. 1985. Fisheries Statistics of Indonesia, 1983. Jakarta, Indonesia.

Jackson, D.M. 1983. The economics of brackishwater aquaculture on the North Coast of Central Java: A production function analysis. Ph.D. Dissertation, University of Hawaii, Hawaii, U.S.A.

Guerrero, C.V. and L.B. Darrah. 1974. Bangus production cost by type of climate. Planning Service, Office of the Secretary, Department of Agriculture, Quezon City, Philippines.

Lee, C.S. 1983. Production and marketing of milkfish in Taiwan: an economic analysis. ICLARM, Manila, Philippines.

Librero, A.R., E.S. Nicolas, E.O. Vasquez and A.M. Nazareno. 1976. An assessment of the fishpond technology and management in the production of milkfish in the Philippines. SEAFDEC and Philippines Council for Agriculture and Research, Los Banos, Laguna, Philippines.

Librero, A.R. and E.S. Nicholas. 1977. Milkfish farming in the Philippines. SEAFDEC and Philippine Council for Agriculture and Resources Research. Los Banos, Laguna, Philippines.

Ramirez, J.L. 1978. Productivity and returns to inputs of fishpen aquaculture in the Philippines. M.S. Thesis, University of the Philippines, Los Banos, Philippines.

Samson, E. 1984. The milkfish industry in the Philippines. In: J.V. Juario, J.R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc., Manila, Philippines.

Shang, Y.C. 1976a. Economics of various management techniques for pond culture of finfish. South China Sea Fisheries Development and Coordinating Programme, FAO, Manila, Philippines.

Shang, Y.C. 1976b. Economic comparison of milkfish farming in Taiwan and the Philippines. Aquaculture 9: 229-236.

Shang, Y.C. 1976c. Indonesia milkfish farming--An economic evaluation. Fish Farming International. December 1976, Vol. 3, No. 4.

Smith, I.R. and K.C. Chong. 1984a. Southeast Asian milkfish culture: Economic status and prospects. In: J.V. Juario, J.R.P. Ferraris and L.V. Benitez (Eds.) Advances in Milkfish Biology and Culture. Island Publishing House, Inc., Manila, Philippines.

Smith, I.R. and K.C. Chong. 1984b. Market constraints inhibit milkfish expansion in Southeast Asia. Aquaculture Magazine, Vol. 10, No. 6.

Taiwan Fisheries Bureau. 1984. Fisheries Yearbook. Taipei, Taiwan.

Wiratno. 1978. An economic analysis of brackishwater pond operation in Central Java, Indonesia. M.S. Thesis, Thammasat University, Bangkok, Thailand.

11. SUMMARY AND RECOMMENDATIONS FOR FUTURE RESEARCH

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11-1. FACTS, THEORIES AND IDEAS

This chapter summarized the facts, theories and ideas presented in the first ten chapters. The previous chapters also indicated major gaps in present knowledge of milkfish. We use these to formulate recommendations for future research helpful to the milkfish industry.

In compiling this book, the editors have identified four significant trends. First, despite over 700 years of commercial-scale culture, our knowledge of milkfish biology and of principles for optimal culture remains fragmentary. Second, prospects are excellent that we will soon establish simple and reliable techniques for routinely spawning captive adult milkfish. This in turn will provide the basis for a stable, reliable supply of fry for the traditional industry, independent of variations in the natural production of larvae. Third, the next few years should also see the development of simple and reliable techniques for successful routine hatching of the eggs obtained from captive spawnings and for larval rearing to the stage at which larvae are now captured from the wild. Fourth, the potential socio-economic impacts of these developments is substantial. The fry capture and distribution components of the traditional industry will probably be most directly affected, but there are also likely to be significant effects on overall industry productivity and costs. Serious efforts must be made to accentuate positive effects.

The following points were made in the first ten chapters. Little is known of the natural life cycle of the milkfish or of its biochemical, physiological, behavioral and ecological properties (Chapter 1). There are at least three statistically distinguishable morphological and genetic intraspecific groups of milkfish distributed across the tropical and subtropical Indo-Pacific Ocean. Despite their range of distribution, levels of differentiation between these groups are small. The differences probably have no practical significance (Chapter 2). Simple and reliable techniques for maturing and inducing spawning in adult fish will soon be available (Chapters 3 and 4). Survival of both fertilized eggs and larvae have been enhanced (Chapter 5). Some improvements have been made in traditional methods of fry collection and handling; rates of loss have decreased (Chapter 6). Knowledge of milkfish nutritional requirements is increasing rapidly, but is still limited. Formulated feeds have been developed which aid in the transition from extensive to intensive farming (Chapter 7). Traditional pond culture methods often expose milkfish to extreme environmental conditions, but the species tolerates and thrives under wide ranges of such conditions (Chapter 8). A variety of diseases afflict milkfish, especially when culture conditions are adverse. Many of these can be treated and managed in fairly straightforward fashion (Chapter 9). Socioeconomic influences upon the traditional industry are complex. Supply and demand are often imbalanced, with adverse impacts on either production or prices or both.

Properties of the industry vary dramatically both between and within countries (Chapter 10).

11-2. RECOMMENDATIONS FOR FUTURE RESEARCH

Chapter 1. Knowledge of the biology of wild stocks should be strengthened. Because of its dependence on wild fry for seed, the milkfish industry is limited to areas with abundant wild populations. Studies are needed on early life history, population dynamics, behavior, physiology and reproduction in the natural environment. Technology for domestication can be improved through a more complete understanding of milkfish natural habits and capacities. Wild populations provide reservoirs of genetic diversity and stocks for replacement of captive fish lost to disease or accident.

Chapter 2. Genetic diversity must be maintained while researching methods for artificial propagation. Further research on diversity should expand the range of sampling sites to include extreme geographic locations (Red Sea, Central America) and should focus on samples obtained from wild stocks. Information is needed on the properties of genetically-diverse wild stocks: growth rates, maturation age and other important features of life histories. Empirical selective breeding programs are needed, using broodstock of genetically diverse origin to reduce sizes/ages of maturity and increase fecundity, growth rates, disease resistance, and adaptability to specific culture environments.

Chapter 3. Further work on reproduction is needed. Induction of maturation and spawning has not yet become routine, nor have such techniques been adopted by the industry. Research should include, but not be limited to, commercial development of experimental techniques for inducing spawnings; improvements in management techniques which reduce time required to reach sizes appropriate for induced spawning; and studies in areas such as reproductive endocrinology and environmental and nutritional factors related to maturation and spawning.

Chapter 4. Artificial propagation should ultimately lead to natural spawnings, maturation at younger ages and expansion of spawning seasons. Advantages of natural spawning, as opposed to strip spawning, include survival of valuable spawners, reduced handling stress, higher fertilization rates and better egg quality. One problem associated with artificial propagation is that milkfish apparently need more than 5 years to reach sexual maturity. Shortening this period by use of either environmental controls or hormone therapy would have an immense impact on the industry. The relationships between sexual maturation and body size or age must be determined. Maturation and spawning have been induced through the application of chronic hormone therapies. This technology should be refined to improve its efficiency. Spawning behavior also deserves further study.

Chapter 5. Cost-effective culture systems for larval milkfish must be developed. Although milkfish larvae are relatively easy to raise compared to many other marine fishes, we lack baseline information essential for developing effective systems. The salient feature of the milkfish industry is the production of protein at low cost. Thus, fry production systems must be highly cost-effective. Hatchery fry must be produced at costs competitive with wild-caught fry. Intensive culture systems, such as those developed in Japan for red sea bream, may not be cost-effective for milkfish. Use of artificial diets to replace live food organisms and extensive pond culture systems should both be evaluated as part of developing effective systems.

Chapter 6. Studies on milkfish fry should emphasize techniques that will maximize use of natural fry production. This can be done by knowledge of the distribution and abundance of natural spawn and of the transport or movements of larvae inshore. Subsequent policies should be developed to protect broodstock and nursery grounds. The degree to which present harvesting affects recruitment rates should be assessed, and appropriate actions taken if there are significant adverse effects. Better methods should be developed in the fry fishery to reduce mortalities incurred during fry storage, transport and acclimation.

Chapter 7. Currently, the majority of milkfish farms depend on natural pond productivity for milkfish growth. Improving pond management techniques is essential if future requirements of the milkfish industry are to be met. In addition, a cheap, complete artificial diet should be developed. A thorough understanding of the digestive system and nutritional requirements at different life stages would be helpful in the development of such diets.

Chapter 8. New pond management strategies need to be adopted in order to compete with the advanced technology used for culture of other aquatic species. One such strategy is the deepwater culture method. Polyculture methods should also be improved to increase production. A better method for overwintering needs to be developed for areas having low winter temperatures.

Chapter 9. Under the extensive culture system, milkfish disease is marginal and does not threaten the industry. Disease prevention is especially critical, however, in intensive culture systems and overwintering ponds.

Chapter 10. Economic analyses of the industry should be conducted on a regular and frequent basis. The profitability of the milkfish industry can be improved if production costs are analyzed and major cost items reduced. Better marketing strategies will increase demand and, consequently, profits. Macro-economic studies should replace the usual micro-economic analyses.

Finally, milkfish will continue to be one of the major finfish species cultured in Asia. The growth of the milkfish industry will depend, however, on the ability to control the life cycle and improve profitability. These factors, in turn, depend on continuing research efforts.